Methods and Compositions for Treating and Preventing Infection Using Human Interferon Regulatory Factor 3

[0001] This application claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Application No. 60/239,936, filed October 13, 2000; and is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of U.S. Non-Provisional Application Serial No. 08/705,771, filed August 30, 1996 (now U.S.Patent 6,054,289), which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Application No. 60/002993, filed August 30, 1995; each of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to a novel human gene encoding a polypeptide which is a member of the Interferon Regulatory Factor family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Interferon Regulatory Factor 3, or "IRF3." This invention also relates to IRF3 polypeptides, as well as vectors, host cells, antibodies directed to IRF3 polypeptides, and the recombinant methods for producing the same. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the immune system, and therapeutic methods, such as gene therapy, for treating, preventing, detecting, and/or diagnosing such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying agonists and antagonists of IRF3 activity.

BACKGROUND OF THE INVENTION

[0003] Identification and sequencing of human genes is a major goal of modern scientific research. For example, by identifying genes and determining their sequences, scientists have been able to make large quantities of valuable human "gene products." These include human insulin, interferon, Factor VIII, tumor necrosis factor, human growth hormone, tissue plasminogen activator, and numerous other compounds. Additionally, knowledge of gene sequences can provide the key to treatment or cure of genetic diseases (such as muscular dystrophy and cystic fibrosis).

[0004] The interferon regulatory factors (IRF) consist of a growing family of related

transcription proteins first identified as regulators of the alpha beta interferon (IFN-alpha/beta) gene promoters, as well as the interferon-stimulated response element (ISRE) of some IFN-stimulated genes. Accordingly, there is a need to provide interferon regulatory factors that are involved in immune responses. Such interferon regulatory factors may be used to make novel agonists or antagonists that increase or decrease the activity of these transcripton factors for diagnosis and therapy of immune system diseases and disorders or to enhance the immune response to infectious agents, particularly viral infections such as HIV infections. There is also a need to provide IRF3 interacting proteins that may be involved in pathological conditions. Such IRF3 interacting proteins may be used, for example, as therapeutics to treat or prevent diseases, disorders or conditions associated with aberrant IRF3 activity.

SUMMARY OF THE INVENTION

[0005] The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of IRF3. Thus, the present invention provides, for example, isolated nucleic acid molecules comprising a polynucleotide encoding the IRF3 transcription factor having the amino acid sequence shown in Figure 1 (SEQ ID NO:2).

[0006] The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells. The invention further provides for the use of such recombinant vectors in the production of IRF3 polypeptides by recombinant techniques.

[0007] The present invention provides for the use of polynucleotides of the invention in gene therapy. In specific embodiments, the present invention provides for the use of polynucleotides of the invention in gene therapy for the treatment and/or amelioration of viral infections. In even more preferred embodiments, the present invention provides for the use of polynucleotides of the invention in gene therapy for the treatment and/or amelioration of HIV infection.

[0008] The invention further provides an isolated IRF3 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

[0009] The present invention also provides diagnostic assays such as quantitative and

diagnostic assays for detecting levels of IRF3 protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of IRF3, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

[0010] The present invention is also directed to methods for enhancing transcription from promoters containing IRF3 binding sites induced by an interferon polypeptide or by viral infection (e.g., interferon alpha, HIV infection) which involves administering to a cell which expresses the IRF3 polypeptide (e.g., a T cell) an effective amount of an IRF3 agonist capable of increasing enhancing transcription from promoters containing IRF3 binding sites.

[0011] Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit transcription from promoters containing IRF3 binding sites can be determined using or routinely modifying reporter assays known in the art, including, for example, those described in Schafer et al., J. Biol Chem. 273:2714 (1998), Lin et al., Mol. Cell. Biol. 19:959 (1999) and herein. Thus, in a further embodiment, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or decreasing transcription from promoters containing IRF3 binding sites in response to interferon treatment, or viral infection The method involves contacting cells expressing IRF3 with the candidate compound (i.e., candidate agonist or antagonist compound), and measuring the IRF3 mediated transcription (e.g., activation of promoters containing IRF3 binding sites, such as, for example, promoters containing interferon sensitive response elements (ISRE) such as the interfreron stimulated gene 15 (ISG15) or promoters containing PRDI-PRDIII elements), and comparing the cellular response to a standard cellular response. The standard cellular response being measured under conditions of interferon treatment or viral infection (e.g., interferon-alpha treatment or HIV infection) in absence of the candidate compound. An increased cellular response over the standard indicates that the candidate compound is an agonist of the IRF3 and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of IRF3. By the invention, a cell expressing the IRF3 polypeptide can be contacted with either an endogenous or exogenously administered interferon (e.g., interferon alpha).

BRIEF DESCRIPTION OF THE FIGURES

[0012] **Figure 1** shows the nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of IRF3. Predicted amino acids from about 1 to about 107 constitute the DNA binding domain (SEQ ID NO:2); amino acids from about 141 to about 147 constitute the nuclear export signal (SEQ ID NO:2); amino acids from about 198 to about 381 constitute the interferon regulatory factor association domain (SEQ ID NO:2); amino acids from about 382 to about 407 constitute the phosphorylation region (SEQ ID NO:2); and amino acids from about 408 to about 427 constitute the autoinhibitory domain (SEQ ID NO:2).

[0013] **Figure 2** shows an analysis of the IRF3 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. The regions were determined by analyzing the amino acid sequence of Figure 1 (SEQ ID NO:2) using the default parameters of the recited computer programs. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues 4-8, 28-33, 66-71, 94-105, 118-128, 132-136, 153-157, 165-168, 173-178, 186-193, 198-202, 233-238, 304-316, 334-340, and 423-427 in Figure 1 (SEQ ID NO:2) correspond to highly antigenic regions of the IRF3 protein.

[0014] A tabular representation of the data summarized graphically in Figure 2 can be found in Table I. In Table I, the columns are labeled with the headings "Res," "Position," and Roman numerals I-XIV. The column headings refer to the Following Features of the amino acid seqeunce presented in Figure 2 and Table I: "Res": amino acid residue of SEQ ID NO:2 and Figure 1; "Position": position of the corresponding residue within SEQ ID NO:2 and Figure 1; "I": Alpha Regions-Garnier-Robson; "II": Alpha Regions-Chou-Fasman; "III": Beta Regsions-Garnier_Robson; "IV": Beta Regions - Chou-Fasman; "V": Turn Regions - Garnier-Robson; "VI": Turn Regions - Chou-Fasman; "VII": Coil Regions - Garnier-Robson; "VIII": Hydrophilicity Plot - Kyte-Doolittle; "IX": Hydrophobicity Plot - Hopp-Woods; "X": Alpha Amphipathic Regions - Eisenberg; "XI": Beta Amphipathic Regions - Eisenberg; "XII": Flexible Regions - Karplus-Schulz; "XIII": Antigenic Index - Jameson-Wolf; "XIV": Surface Probability Plot - Emini.

DETAILED DESCRIPTION OF THE INVENTION

[0015] In accordance with an aspect of the present invention, there are provided

isolated nucleic acids (polynucleotides) which code for mature polypeptides having the deduced amino acid sequences shown in the Figures 1 and 2 or for the mature polypeptides encoded by the cDNA of the clone deposited as ATCC Deposit No. 97242 on Aug. 15, 1995 with ATCC, 10801 University Boulevard, Manassas, Va. 20110-2209.

[0016] The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding IRF3, such as, for example, polynucleotides having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1). The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding an IRF3 polypeptide having the amino acid sequence shown in Figure 1 (SEQ ID NO:2).

[0017] The human interferon regulatory factor IRF3 gene shows strong homology to a group of transcription factors including IRF1 (Interferon Regulatory factor 1) and IRF2 (interferon Regulatory factor 2) which are important in mediating the transcriptional activation of interferon-alpha and -beta induced genes. It is possible that this gene also is important in mediating the transcriptional activation properties of interferon and that this factor may have some of the properties associated with interferon such as anti-viral activity. The human interferon regulatory factor IRF3 is potentially important in regulating the transcriptional activation of interferon-alpha and -beta genes. IRF3 may also be important in mediating the transcriptional activation properties of interferon. The IRF3 polypeptide may be employed as an anti-viral agent. The administration of the IRF3 gene and its gene product may be employed to enhance the expression of interferon which has many medically important uses. The IRF3 gene was isolated from a human adult retina library.

<u>IRF3 Nucleic Acid Molecules</u>

[0018] The determined nucleotide sequence of IRF3 (Figure 1; SEQ ID NO:1) contains an open reading frame encoding a protein of 427 amino acid residues, with a deduced molecular weight of about 47.2 kDa. The amino acid sequence of the predicted IRF3 transcription factor is shown in SEQ ID NO:2 from amino acid residue 1 to residue 427.

[0019] The present invention provides a nucleotide sequence encoding the IRF3 polypeptide having the amino acid sequence shown in Figure 1. By the IRF3 protein having the amino acid sequence shown in Figure 1 is meant the form(s) of the IRF3 transcription factor predicted by computer analysis or produced by expression of the

coding sequence shown in Figure 1 in a mammalian cell (e.g., COS cells, as described below).

[0020] The predicted IRF3 polypeptide, comprises about 184 amino acids. However, as one of oridinary skill in the art would appreciate, the actual IRF3 polypeptide may be anywhere in the range of 417 to 437 amino acids due to the possibilities of sequencing errors as well as the variability of cleavage sites for leaders in different known proteins. It will further be appreciated that, the domains described herein have been predicted based on experiments with deletion mutants, and accordingly, that depending on the deletion mutants used for identifying various functional domains, the exact "address" of, for example, the DNA binding domain, interferon association domain, nuclear export signal, phosphorylation domain, and autoinhibitory domain of IRF3 may differ slightly from the predicted locations. For example, the exact location of the IRF3 extracellular domain in Figure 1 (SEQ ID NO:2) may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the mutants used to define the domain. In any event, as discussed in more detail below, the invention further provides polypeptides having various residues deleted from the N-terminus and/or C-terminus of the complete IRF3 polypeptide.

[0021] As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

[0022] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced naturally, recombinantly or synthetically. However, a nucleic acid molecule contained in a

clone that is a member of a mixed clone library (e.g., a genomic or cDNA library) and that has not been isolated from other clones of the library (e.g., in the form of a homogeneous solution containing the clone without other members of the library) or a chromosome isolated or removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), or a preparation of randomly sheared or genomic DNA cut with one or more restriction enzymes, is not "isolated" for the purposes of this invention.

[0023] Isolated nucleic acid molecules of the present invention include DNA molecules comprising the open reading frame (ORF) shown in Figure 1 (SEQ ID NO:1); DNA molecules comprising the coding sequence for the complete (full-length) IRF3 protein shown in Figure 1 (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above, but which, due to the degeneracy of the genetic code, still encode the IRF3 protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

[0024] The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), or a nucleic acid molecule having a sequence complementary thereto. Such isolated molecules, particularly DNA molecules, are useful, for example, as probes for gene mapping by *in situ* hybridization with chromosomes, and for detecting expression of the IRF3 gene in human tissue, for instance, by Northern blot analysis.

[0025] The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated DNA molecule having the nucleotide sequence of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) is intended DNA fragments at least about 15nt, and more preferably at least about 20 nt, at least about 24 nt, still more preferably at least about 30 nt, at least about 35 nt, and even more preferably, at least about 40 nt, at least about 45 nt, at least about 50 nt, at least about 55 nt, at least about 60 nt, at least about 65 nt, at least about 70 nt, at least about 75 nt, at least about 100 nt, at least about 150 nt, at least about 200 nt, at least about 250 nt, at least about 300 nt, at least about 350 nt, at least about 400 nt, at least about 450 nt, at least about 500 nt in length which are useful, for example, as diagnostic probes and primers as discussed herein. Of course, larger fragments 500-1426 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of

the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1), or the complementary strand thereto. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1). In this context "about" includes the particularly recited size, and sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In specific embodiments, the fragments of the invention comprise, or alternatively consist of, nucleotides 59-70, 128-145, 242-259, 226-361, 398-430, 503-517, 539-547, 560-577, 602-625, 638-652, 733-750, 956-994, and/or 1313-1327 of Figure 1 (SEQ ID NO:1) or the complementary strand thereto. Polypeptides encoded by these polynucleotide are also encompassed.

[0026] Representative examples of IRF3 polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 23, 24 to 46, 47 to 79, 80 to 111, 112 to 143, 144 to 175, 176 to 207, 208 to 239, 240 to 271, 272 to 303, 304 to 335, 336 to 367, 368 to 400, 401 to 433, 434 to 466, 467 to 487, 488 to 517, 518 to 547, 548 to 577, 578 to 607, 608 to 637, 638 to 668, 669 to 699, 700 to 729, 730 to 760, 761 to 791, 792 to 821, 822 to 852, 853 to 883, 884 to 913, 914 to 944, 945 to 975, 976 to 1005, 1006 to 1036, 1037 to 1067, 1097 to 1127, 1128 to 1158, 1159-1189, 1190-1228, 1229-1267, 1268-1297, 1298-1327, 1328-1366, 1367-1405, and/or 1406-1426 of Figure 1 (SEQ ID NO:1), or the complementary strand thereto. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0027] In specific embodiments, the polynucleotide fragments of the invention comprise, or alternatively, consist of, a sequence from nucleotide 1 to 407, of Figure 1 (SEQ ID NO:1), or the complementary strand thereto.

[0028] Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates an IRF3 functional activity. By a polypeptide demonstrating an IRF3 "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) IRF3 protein. Such functional activities include, but are not limited to, biological activity, antigenicity (ability to bind (or compete with an IRF3 polypeptide for binding) to an anti-IRF3 antibody), immunogenicity (ability to generate antibody which binds to an IRF3 polypeptide), ability

to interact with othe interferon regulatory factors (e.g., IRF7) or other transcription factors (e.g., RelA, Creb binding protein (CBP)), and ability to bind to an promoter containing an IRF3 binding site (e.g., ISRE containing promoter (ISG15 promoter) or an PRDI-PRDIII containing promoter (Interferon-alpha or Interferon-beta promoters)).

[0029] The functional activity of IRF3 polypeptides, fragments, variants, derivatives, and analogs thereof, can be assayed by various methods.

[0030] For example, in one embodiment where one is assaying for the ability to bind or compete with full-length IRF3 polypeptides for binding to anti-IRF3 antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0031] In another embodiment, where an IRF3 target gene is identified (i.e., a gene which is regulated in part or completely by IRF3), or the ability of a polypeptide fragment, variant or derivative of the invention to interact with other transcription factors, binding can be assayed by means well-known in the art, such as, for example, immunoprecipitation, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., *et al., Microbiol. Rev. 59*:94-123 (1995). In another embodiment, physiological correlates of IRF3 binding to its substrates (transcription) can be assayed.

[0032] In addition, assays described herein (and otherwise known in the art may routinely be applied to measure the ability of IRF3 polypeptides and fragments, variants derivatives and analogs thereof to elicit IRF3 related biological activity. For example,

techniques described herein and otherwise known in the art may be applied or routinely modified to assay for the ability of the compositions of the invention (e.g., fusion proteins comprising a portion of the DNA binding portion of IRF3 (e.g. a amino acid residues 1-133 of SEQ ID NO 2) and a transactivation domain of another protein (e.g., amino acid residues of 397-550 of the RelA/p65 protein as decribed in Schafer et al, J. Biol. Chem. 273:2714 (1998)) to activate transcription of the interferon-alpha or interferon-beta genes.

[0033] Other methods will be known to the skilled artisan and are within the scope of the invention.

[0034] Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding a member selected from the group: a polypeptide comprising or alternatively, consisting of, the IRF3 DNA binding domain (amino acid residues from about 1 to about 107 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, the IRF3 nuclear export signal (amino acid residues from about 141 to about 147 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of the IRF3 interferon regulatory factor association domain (amino acid residues from about 198 to about 381 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, the IRF3 phosphorylation region (amino acid residues from about 382 to about 407 in Figure 1(SEQ ID NO:2) ; and a polypeptide comprising, or alternatively consisting of, the IRF3 autoinhibitory domain (amino acid residues from about 408 to about 427 in Figure 1(SEQ ID NO:2). Since the locations of these domains have been determined using IRF3 deletion mutants, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 amino acid residues) depending on the deletion mutants used to define each domain.

[0035] Preferred nucleic acid fragments of the invention encode a full-length IRF3 polypeptide lacking the nucleotides encoding the amino terminal methionine in Figure 1 (SEQ ID NO:1), as it is known that the methionine is cleaved naturally and such sequences may be useful in genetically engineering IRF3 expression vectors. Polypeptides encoded by such polynucleotides are also contemplated by the invention.

[0036] Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the IRF3 transcription factor proteins. In particular, such nucleic acid fragments of the present invention include

nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 4 to about 8 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 28 to about 33 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 66 to about 71 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 94 to about 105 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 118 to about 128 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 132 to about 136 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 153 to about 157 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 165 to about 168 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 173 to about 178 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 186 to about 193 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 198 to about 202 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 233 to about 238 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 304 to about 316 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 423 to about 427 in Figure 1 (SEQ ID NO:2). In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. The inventors have determined that the above polypeptide fragments are antigenic regions of the IRF3 proteins. Methods for determining other such epitope-bearing portions of the IRF3 proteins are described in detail below.

[0037] In additional embodiments, the polynucleotides of the invention encode functional attributes of IRF3. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of IRF3.

[0038] The data representing the structural or functional attributes of IRF3 set forth in Figure 2 and/or Table I, as described above, was generated using the various modules and

algorithms of the DNA*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, XI, XIII and XIV of Table I can be used to determine regions of IRF3 which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, XI, XIII and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0039] Certain preferred regions in these regards are set out in Figure 2, but may, as shown in Table I, be represented or identified by using tabular representations of the data presented in Figure 2. The DNA*STAR computer algorithm used to generate Figure 2 (set on the original default parameters) was used to present the data in Figure 2 in a tabular format (See Table I). The tabular format of the data in Figure 2 may be used to easily determine specific boundaries of a preferred region.

[0040] The above-mentioned preferred regions set out in Figure 2 and in Table I, include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequences set out in Figure 1. As set out in Figure 2 and in Table I, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions, Hopp-Woods hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Jameson-Wolf regions of high antigenic index and Emini surface-forming regions.

Table I

Res	Pos	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	A	A	.	.	.	.	.	-0.66	0.60	.	.	.	-0.60	0.52
Leu	2	A	A	.	.	.	.	.	-0.61	0.67	*	.	.	-0.60	0.41
Gln	3	A	A	.	.	.	.	.	-0.22	0.67	*	.	.	-0.60	0.32
Met	4	A	A	.	.	.	.	.	-0.50	0.64	*	*	.	-0.60	0.56
Ala	5	A	A	.	.	.	.	.	-0.41	0.60	*	.	.	-0.60	0.36
Gly	6	A	.	.	.	.	.	.	0.19	0.30	*	.	.	0.18	0.28
Gln	7	.	.	.	.	T	.	.	1.00	0.30	*	*	F	1.01	0.49
Cys	8	.	.	.	.	.	.	C	1.00	0.09	.	.	F	1.09	0.79
Ser	9	.	.	.	.	.	T	C	1.36	-0.41	.	.	F	2.32	1.38
Gln	10	.	.	.	.	T	T	.	1.24	-0.09	.	.	F	2.80	1.24
Asn	11	.	.	.	.	T	T	.	1.59	0.30	.	.	F	1.92	2.01
Glu	12	.	.	.	.	T	T	.	1.29	-0.27	.	.	F	2.24	2.51
Tyr	13	.	A	.	.	T	.	.	1.14	-0.27	*	.	F	1.56	1.94
Phe	14	.	A	.	.	T	.	.	0.63	0.01	*	.	.	0.38	0.99
Asp	15	A	A	.	.	.	.	.	0.60	0.30	*	.	.	-0.30	0.47
Ser	16	A	A	.	.	.	.	.	0.01	0.80	*	.	.	-0.60	0.41
Leu	17	A	A	.	.	.	.	.	-0.66	0.54	*	*	.	-0.60	0.48
Leu	18	A	A	.	.	.	.	.	-1.30	0.33	*	*	.	-0.30	0.15
His	19	A	A	.	.	.	.	.	-0.81	1.01	*	*	.	-0.60	0.08
Ala	20	.	A	.	.	T	.	.	-1.48	1.06	.	*	.	-0.20	0.15
Cys	21	.	A	.	.	T	.	.	-1.18	0.94	.	*	.	-0.20	0.10
Ile	22	A	.	.	.	.	.	.	-1.18	0.66	*	*	.	-0.40	0.12
Pro	23	.	.	.	.	T	.	.	-0.26	0.84	*	*	.	0.00	0.10
Cys	24	.	.	.	.	T	.	.	-0.89	0.34	.	*	.	0.30	0.37
Gln	25	.	.	.	.	T	.	.	-0.60	0.34	.	*	.	0.30	0.28
Leu	26	.	.	.	.	T	.	.	-0.23	0.04	*	*	.	0.55	0.25
Arg	27	.	.	.	.	T	.	.	0.66	0.00	*	*	.	0.80	0.62
Cys	28	.	.	.	.	T	.	.	0.56	-0.17	.	*	.	1.65	0.57
Ser	29	.	.	.	.	T	T	.	1.01	-0.09	.	*	F	2.40	1.00
Ser	30	.	.	.	.	T	T	.	0.80	-0.34	.	*	F	2.50	0.79
Asn	31	.	.	.	.	T	T	.	0.80	0.09	*	*	F	1.80	2.28
Thr	32	.	.	.	.	.	T	C	0.38	0.20	*	*	F	1.35	1.41
Pro	33	.	.	.	.	.	T	C	0.38	0.30	.	.	F	1.10	1.51
Pro	34	.	.	.	.	T	T	.	0.68	0.49	.	.	F	0.60	0.50
Leu	35	.	.	.	.	T	T	.	1.09	0.49	.	.	F	0.35	0.61
Thr	36	.	.	.	.	T	T	.	0.84	0.00	.	.	.	0.50	0.77
Cys	37	.	.	B	.	.	.	.	0.49	0.33	*	.	.	-0.10	0.78
Gln	38	.	.	B	.	.	.	.	0.70	0.47	.	.	.	-0.40	0.51
Arg	39	.	.	.	.	T	.	.	0.32	0.19	.	.	.	0.30	0.56
Tyr	40	.	.	.	.	T	.	.	0.83	0.20	.	.	.	0.45	1.06
Cys	41	.	.	.	.	T	.	.	0.29	0.01	*	.	.	0.30	0.82
Asn	42	.	.	.	.	T	T	.	0.64	0.26	*	.	.	0.50	0.31
Ala	43	.	.	.	.	T	T	.	0.64	0.74	*	.	.	0.20	0.29
Ser	44	.	.	.	.	T	T	.	0.23	0.39	*	.	.	0.50	0.86
Val	45	.	.	.	.	.	T	C	-0.38	0.20	*	*	F	0.45	0.72
Thr	46	.	.	B	B	.	.	.	0.33	0.44	*	*	F	-0.45	0.53
Asn	47	.	.	B	B	.	.	.	-0.01	-0.06	.	*	F	0.66	0.79
Ser	48	.	.	.	B	.	.	C	0.27	-0.01	*	*	F	1.22	1.05
Val	49	.	.	.	B	T	.	.	0.57	-0.17	*	.	F	1.63	1.05
Lys	50	.	.	.	B	T	.	.	0.83	-0.26	*	*	F	1.84	1.05
Gly	51	.	.	.	.	.	T	C	0.26	-0.16	*	.	F	2.10	0.79
Thr	52	.	.	.	.	T	T	.	-0.56	0.14	*	.	F	1.49	0.75
Asn	53	.	.	.	.	.	T	C	-0.54	0.19	.	.	F	1.08	0.31
Ala	54	.	.	B	.	.	T	.	0.00	1.10	*	.	.	0.22	0.33
Ile	55	.	.	B	B	.	.	.	-0.71	1.16	.	.	.	-0.39	0.33
Leu	56	.	.	B	B	.	.	.	-1.18	1.24	.	.	.	-0.60	0.11
Trp	57	.	.	B	B	.	.	.	-1.21	1.53	.	.	.	-0.60	0.09
Thr	58	.	.	B	B	.	.	.	-2.02	1.46	.	.	.	-0.60	0.13
Cys	59	A	.	.	B	.	.	.	-1.73	1.46	.	.	.	-0.60	0.13
Leu	60	A	.	.	B	.	.	.	-1.66	1.16	.	.	.	-0.60	0.16

Gly	61	.	.	.	B	T	.	.	-1.73	0.93	.	.	.	-0.20	0.09
Leu	62	.	.	.	B	.	.	C	-2.33	1.13	.	.	.	-0.40	0.12
Ser	63	.	.	.	B	.	.	C	-2.32	1.24	.	*	.	-0.40	0.10
Leu	64	A	.	.	B	.	.	.	-2.47	0.94	.	*	.	-0.60	0.14
Ile	65	A	.	.	B	.	.	.	-2.24	1.20	.	*	.	-0.60	0.14
Ile	66	A	.	.	B	.	.	.	-2.76	1.01	.	.	.	-0.60	0.10
Ser	67	A	.	.	B	.	.	.	-2.64	1.27	.	*	.	-0.60	0.09
Leu	68	A	.	.	B	.	.	.	-3.20	1.37	.	.	.	-0.60	0.12
Ala	69	A	.	.	B	.	.	.	-3.20	1.33	.	.	.	-0.60	0.12
Val	70	A	.	.	B	.	.	.	-2.91	1.33	.	.	.	-0.60	0.08
Phe	71	A	.	.	B	.	.	.	-2.72	1.56	.	.	.	-0.60	0.09
Val	72	A	.	.	B	.	.	.	-3.23	1.66	.	.	.	-0.60	0.08
Leu	73	A	.	.	B	.	.	.	-3.23	1.84	*	*	.	-0.60	0.09
Met	74	A	.	.	B	.	.	.	-2.53	1.89	*	.	.	-0.60	0.08
Phe	75	A	.	.	B	.	.	.	-1.63	1.10	*	.	.	-0.60	0.22
Leu	76	A	.	.	B	.	.	.	-1.82	0.46	*	.	.	-0.60	0.52
Leu	77	A	.	.	B	.	.	.	-1.27	0.46	*	.	.	-0.60	0.37
Arg	78	A	.	.	B	.	.	.	-0.76	0.23	*	.	F	-0.15	0.57
Lys	79	A	.	.	B	.	.	.	-0.16	-0.17	*	.	F	0.45	0.93
Ile	80	A	.	.	B	.	.	.	0.33	-0.86	*	.	F	0.90	1.96
Ser	81	.	.	.	.	.	T	C	0.33	-1.11	*	.	F	1.50	1.55
Ser	82	.	.	.	.	.	T	C	1.19	-0.43	*	.	F	1.05	0.64
Glu	83	A	.	.	.	.	T	.	1.08	-0.43	*	.	F	1.00	1.82
Pro	84	A	.	.	.	.	T	.	1.03	-1.11	*	*	F	1.30	2.27
Leu	85	A	A	.	.	.	.	.	1.22	-1.50	*	*	F	0.90	2.93
Lys	86	A	A	.	.	.	.	.	1.57	-1.10	*	*	F	0.90	1.46
Asp	87	A	A	.	.	.	.	.	1.87	-1.10	*	*	F	0.90	1.89
Glu	88	A	A	.	.	.	.	.	1.56	-1.13	*	*	F	0.90	3.69
Phe	89	A	A	.	.	.	.	.	1.42	-1.33	*	*	F	1.15	2.66
Lys	90	A	A	.	.	.	.	.	1.93	-0.90	*	*	F	1.40	1.58
Asn	91	.	.	.	.	T	T	.	1.54	-0.51	*	*	F	2.45	1.22
Thr	92	.	.	.	.	.	T	C	0.73	-0.09	*	.	F	2.20	1.40
Gly	93	.	.	.	.	T	T	.	-0.08	-0.19	*	*	F	2.50	0.58
Ser	94	.	.	.	.	.	T	C	0.28	0.50	*	.	F	1.15	0.30
Gly	95	.	.	.	.	.	.	C	-0.37	0.53	*	.	F	0.70	0.20
Leu	96	.	A	.	.	.	.	C	-0.96	0.66	*	.	F	0.25	0.20
Leu	97	.	A	.	.	.	.	C	-0.64	0.73	.	.	.	-0.15	0.15
Gly	98	A	A	.	.	.	.	.	-1.19	0.74	.	*	.	-0.60	0.25
Met	99	A	A	.	.	.	.	.	-0.89	1.00	.	*	.	-0.60	0.21
Ala	100	A	A	.	.	.	.	.	-1.36	0.31	.	*	.	-0.30	0.43
Asn	101	A	A	.	.	.	.	.	-0.54	0.31	.	*	.	-0.30	0.36
Ile	102	A	A	.	.	.	.	.	0.31	-0.11	.	*	.	0.30	0.62
Asp	103	A	A	.	.	.	.	.	0.36	-0.73	.	*	.	0.75	1.23
Leu	104	A	A	.	.	.	.	.	1.07	-0.84	.	*	F	1.24	1.03
Glu	105	A	A	.	.	.	.	.	1.34	-1.24	.	*	F	1.58	2.87
Lys	106	A	A	.	.	.	.	.	1.00	-1.44	.	.	F	1.92	2.48
Ser	107	.	.	.	.	.	T	C	1.89	-1.01	.	.	F	2.86	2.98
Arg	108	.	.	.	.	T	T	.	1.89	-1.70	.	*	F	3.40	2.87
Thr	109	.	.	.	.	T	T	.	1.81	-1.70	*	.	F	3.06	2.49
Gly	110	A	.	.	.	.	T	.	0.92	-1.01	*	*	F	2.32	1.30
Asp	111	A	.	.	.	.	.	.	0.07	-0.71	*	*	F	1.63	0.47
Glu	112	A	.	.	.	.	.	.	0.16	-0.03	.	*	.	0.84	0.27
Ile	113	.	.	B	.	.	.	.	0.16	-0.09	.	*	.	0.50	0.42
Ile	114	.	.	B	.	.	.	.	0.12	-0.51	.	.	.	1.04	0.49
Leu	115	.	.	B	.	.	T	.	-0.34	-0.09	.	.	.	1.18	0.28
Pro	116	.	.	.	.	.	T	C	-0.34	0.60	.	.	F	0.87	0.33
Arg	117	.	.	.	.	T	T	.	-0.59	-0.09	*	.	F	2.21	0.81
Gly	118	.	.	.	.	.	T	C	-0.01	-0.01	*	.	F	2.40	1.54
Leu	119	.	.	.	B	.	.	C	0.02	-0.21	*	*	.	1.61	1.44
Glu	120	.	.	.	B	.	.	C	0.83	0.00	*	.	.	0.62	0.54
Tyr	121	.	.	.	B	T	.	.	1.04	0.00	*	.	.	0.58	0.95
Thr	122	A	.	.	B	.	.	.	0.27	-0.43	*	.	.	0.69	2.00
Val	123	A	.	.	B	.	.	.	0.30	-0.54	.	.	.	0.60	0.62
Glu	124	A	.	.	B	.	.	.	0.44	-0.06	.	*	.	0.30	0.57

Glu	125	A	.	.	.	.	.	.	0.44	-0.24	.	.	.	0.50	0.21
Cys	126	A	.	.	.	.	.	.	0.69	-0.73	.	.	.	0.80	0.49
Thr	127	A	.	.	.	.	.	.	0.33	-1.37	.	.	.	0.80	0.48
Cys	128	A	.	.	.	.	T	.	0.30	-0.80	.	*	.	1.00	0.15
Glu	129	A	.	.	.	.	T	.	0.34	-0.11	.	.	.	0.70	0.19
Asp	130	A	.	.	.	.	T	.	0.04	-0.69	.	*	.	1.00	0.27
Cys	131	A	.	.	.	.	T	.	0.76	-0.79	.	*	.	1.00	0.67
Ile	132	A	.	.	.	.	.	.	0.86	-1.36	*	*	F	1.25	0.77
Lys	133	A	.	.	.	.	.	.	1.57	-0.93	*	*	F	1.55	0.71
Ser	134	A	.	.	.	.	.	.	0.71	-0.93	*	*	F	2.00	2.66
Lys	135	.	.	.	.	.	.	C	0.71	-0.86	*	*	F	2.50	2.82
Pro	136	.	.	.	.	T	.	.	1.08	-1.54	*	*	F	3.00	2.35
Lys	137	.	.	.	.	T	.	.	1.97	-1.16	.	*	F	2.70	2.35
Val	138	.	.	.	.	T	.	.	1.89	-1.54	.	*	F	2.65	1.97
Asp	139	.	.	.	.	T	T	.	1.52	-1.04	.	*	F	2.80	1.73
Ser	140	.	.	.	.	T	T	.	0.78	-0.90	.	*	F	2.60	0.46
Asp	141	.	.	.	.	T	T	.	0.78	-0.11	.	*	F	2.25	0.54
His	142	.	.	.	.	T	T	.	-0.08	-0.33	.	*	F	2.50	0.50
Cys	143	.	.	.	.	T	.	.	0.57	0.36	.	*	.	1.30	0.31
Phe	144	.	.	.	.	.	.	C	-0.02	0.40	.	.	.	0.55	0.29
Pro	145	.	.	.	.	.	.	C	-0.32	0.90	.	.	.	0.30	0.21
Leu	146	.	A	.	.	.	.	C	-0.32	1.01	.	.	.	-0.15	0.39
Pro	147	A	A	.	.	.	.	.	-0.29	0.44	.	.	.	-0.60	0.78
Ala	148	A	A	.	.	.	.	.	0.03	-0.34	.	.	.	0.30	0.88
Met	149	A	A	.	.	.	.	.	0.14	-0.34	.	.	.	0.45	1.05
Glu	150	A	A	.	.	.	.	.	0.04	-0.53	.	.	.	0.60	0.69
Glu	151	A	A	.	.	.	.	.	-0.03	-0.47	*	.	F	0.45	0.98
Gly	152	A	.	.	B	.	.	.	-0.63	-0.29	*	.	F	0.45	0.70
Ala	153	A	.	.	B	.	.	.	-0.90	-0.21	*	.	F	0.45	0.33
Thr	154	A	.	.	B	.	.	.	-0.61	0.43	*	.	.	-0.60	0.14
Ile	155	A	.	.	B	.	.	.	-0.92	0.91	.	.	.	-0.60	0.21
Leu	156	A	.	.	B	.	.	.	-0.88	0.97	.	.	.	-0.60	0.30
Val	157	A	.	.	B	.	.	.	-0.84	0.47	.	*	.	-0.60	0.41
Thr	158	A	.	.	B	.	.	.	-0.26	0.47	.	*	F	-0.45	0.84
Thr	159	.	.	.	B	T	.	.	0.06	0.19	.	.	F	0.74	1.64
Lys	160	.	.	.	B	T	.	.	0.70	-0.50	.	.	F	1.68	3.70
Thr	161	.	.	.	.	T	.	.	0.84	-0.39	*	.	F	2.22	4.02
Asn	162	.	.	.	.	T	T	.	1.74	-0.30	*	*	F	2.76	1.49
Asp	163	.	.	.	.	T	T	.	1.76	-0.79	*	.	F	3.40	1.49
Tyr	164	.	.	.	.	T	T	.	1.26	-0.40	*	.	F	2.76	1.39
Cys	165	.	.	.	.	T	T	.	1.00	-0.20	*	.	.	2.12	0.71
Lys	166	.	.	.	.	T	.	.	0.72	-0.17	*	.	.	1.58	0.66
Ser	167	.	.	.	.	.	.	C	0.13	0.33	*	.	.	0.44	0.42
Leu	168	A	A	.	.	.	.	.	-0.68	0.07	*	.	.	-0.30	0.80
Pro	169	A	A	.	.	.	.	.	-0.73	0.19	*	.	.	-0.30	0.33
Ala	170	A	A	.	.	.	.	.	-0.66	0.57	*	.	.	-0.60	0.33
Ala	171	A	A	.	.	.	.	.	-1.01	0.69	*	.	.	-0.60	0.40
Leu	172	A	A	.	.	.	.	.	-0.71	0.49	.	.	.	-0.60	0.38
Ser	173	A	A	.	.	.	.	.	-0.79	0.06	.	.	.	-0.30	0.65
Ala	174	A	A	.	.	.	.	.	-0.58	0.24	*	.	.	-0.30	0.45
Thr	175	A	A	.	.	.	.	.	0.06	-0.26	*	.	F	0.45	0.94
Glu	176	A	A	.	.	.	.	.	0.34	-0.94	*	.	F	0.90	1.41
Ile	177	A	A	.	.	.	.	.	0.27	-0.94	*	.	F	0.90	1.86
Glu	178	A	A	.	.	.	.	.	0.27	-0.76	*	*	F	0.75	0.91
Lys	179	A	A	.	.	.	.	.	0.27	-0.86	*	*	F	0.75	0.70
Ser	180	A	A	.	.	.	.	.	0.69	-0.36	.	*	F	0.60	1.01
Ile	181	A	.	.	.	.	.	.	0.30	-1.04	.	*	F	1.10	1.14
Ser	182	A	.	.	.	.	.	.	0.80	-0.61	.	*	.	0.80	0.73
Ala	183	A	.	.	.	.	.	.	0.41	-0.19	.	*	.	0.50	0.70
Arg	184	A	.	.	.	.	.	.	-0.02	-0.14	.	*	.	0.65	1.27

[0041] In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the complementary strand of nucleotides 59-70, 128-145, 242-259, 226-361, 398-430, 503-517, 539-547, 560-577, 602-625, 638-652, 733-750, 956-994, and/or 1313-1327 of SEQ ID NO:1. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C. Polypeptides encoded by these nucleic acids are also encompassed by the invention.

[0042] By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful, for example, as diagnostic probes and primers as discussed above and in more detail below. By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1). In this context "about" includes the particularly recited size, and sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0043] In specific embodiments, the polynucleotides of the invention are less than 110000 kb, 50000 kb, 10000 kb, 1000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, 7.5 kb, or 5 kb in length.

[0044] In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of IRF3 coding sequence, but consist of less than or equal to 100 kb, 75 kb, 50 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, or 5 kb of genomic DNA that flanks the 5' or 3' coding nucleotide set forth in Figure 1 (SEQ ID NO:1). In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of IRF3 and/or coding

sequence, but do not comprise all or a portion of any IRF3 intron. In another embodiment, the nucleic acid comprising IRF3 coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the IRF3 gene in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[0045] As indicated, nucleic acid molecules of the present invention which encode an IRF3 polypeptide may include, but are not limited to, the coding sequence for the full-length polypeptide, by itself or together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*:767-778 (1984). As discussed below, other such fusion proteins include the IRF3 transcription factor fused to Fc at the N- or C-terminus.

[0046] The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs, or derivatives of the IRF3 transcription factor. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

[0047] Such variants include those produced by nucleotide substitutions, deletions or

additions which may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions, or additions. Especially preferred among these are silent substitutions, additions, and deletions, which do not alter the properties and activities of the IRF3 transcription factor or portions thereof. Also especially preferred in this regard are conservative substitutions.

[0048] Further embodiments of the invention include isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence at least 80%, 85%, or 90% identical, and more preferably at least 95%, 96%, 97%, 98%, or 99% identical to: (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence shown in Figure 1 (SEQ ID NO:2); (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in Figure 1 (SEQ ID NO: 2), but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions 1 to 427 in Figure 1 (SEQ ID NO:2); (d) a nucleotide sequence encoding the IRF3 DNA binding domain; (e) a nucleotide sequence encoding the IRF3 nuclear export signal; (f) a nucleotide sequence encoding the IRF3 interferon regulatory factor association domain; (g) a nucleotide sequence encoding the IRF3 transcription factor phosphorylation domain; (h) a nucleotide sequence encoding the IRF3 autoinhibiotry domain; (i) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions 1 to 407 in Figure 1 (SEQ ID NO:2); and (j) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h) or (i) above. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0049] By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding an IRF3 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five mismatches per each 100 nucleotides of the reference nucleotide sequence encoding the IRF3 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference

sequence. These mismatches of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The reference (query) sequence may be the entire IRF3 encoding nucleotide sequence shown in Figure 1 (SEQ ID NO:1), or any IRF3 polynucleotide fragment (e.g., a polynucleotide encoding the amino acid sequence of any of the IRF3 N- and/or C- terminal deletions described herein), variant, derivative or analog, as described herein.

[0050] As a practical matter, whether any particular nucleic acid molecule is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0051] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not

account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0052] The present application is directed to nucleic acid molecules comprising, or alternatively consisting of a nucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence for example, shown in Figure 1 (SEQ ID NO:1), irrespective of whether they encode a polypeptide having IRF3 biological activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having IRF3 functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a

polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having IRF3 biological activity include, *inter alia:* (1) isolating the IRF3 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the IRF3 transcription factor gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting IRF3 transcription factor mRNA expression in specific tissues.

[0053] Preferred, however, are nucleic acid molecules comprising, or alternatively consisting of, a nucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to for example, the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), which do, in fact, encode a polypeptide having IRF3 functional activity. By "a polypeptide having IRF3 functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the IRF3 transcription factor of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay.

[0054] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to, for example, the nucleic acid shown in Figure 1 (SEQ ID NO:1), will encode a polypeptide "having IRF3-short functional activity." Similarly, a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to, for example, a nucleic acid sequence shown in Figure 1will encode a polypeptide "having IRF3 functional activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing a biological assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having IRF3 functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

[0055] For example, guidance concerning how to make phenotypically silent amino

acid substitutions is provided in J.U. Bowie *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

IRF3 Polynucleotide assays

[0056] This invention is also related to the use of IRF3 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a normal and mutated form of IRF3 associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or infection or susceptibility to a disease or infection which results from under-expression over-expression or altered expression of IRF3 (or a soluble form thereof), such as, for example, viral infections, and autoimmune diseases.

[0057] Individuals carrying mutations in the IRF3 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a biological sample from a patient (e.g., a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material). The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki *et al., Nature 324*:163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding IRF3 can be used to identify and analyze IRF3 expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled IRF3 RNA or alternatively, radiolabeled IRF3 antisense DNA sequences. Perfectly matched sequences can routinely be distinguished from mismatched duplexes by techniques known in the art, such as, for example, RNase A digestion or by differences in melting temperatures.

[0058] Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-

stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

[0059] Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis using techniques known in the art. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al., Science 230*:1242 (1985)).

[0060] Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and Sl protection or the chemical cleavage method (e.g., Cotton *et al., Proc. Natl. Acad. Sci. USA 85*: 4397-4401 (1985)).

[0061] Thus, the detection of a specific DNA sequence may be achieved by methods which include, but are not limited to, hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

[0062] In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

[0063] The invention also encompasses isolated nucleic acids encoding the above-described IRF3 polypeptides and proteins. Such polynucleotide sequences can routinely be determined using techniques known in the art. For example, the amino acid sequence of the IRF3 polypeptides of the invention can be routinely determined using techniques known in the art, such as via the Edman degradation technique. (See, e.g., Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for polynucleotide sequences encoding IRF3 polypeptides. Screening may be accomplished, for example, by standard hybridization or PCR techniques. For example, polynucleotides encoding IRF3 polypeptides of the invention may be isolated by techniques known in the art, such as, for example, by performing PCR using two degenerate oligonucleotide primer pools designed

on the basis of amino acid sequence of the IRF3 polypeptide of interest. Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (See, e.g., Ausubel, supra., and PCR Protocols: A Guide to Methods and Applications, 1990, Innis, M. et al., eds. Academic Press, Inc., New York). The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from, for example, human or non-human cell lines or tissue, such as B cells, known or suspected to express an IRF3 polypeptide.

[0064] The PCR product may be subcloned and sequenced to ensure that the amplified sequences encode an IRF3 polypeptide. The PCR fragment may then be used to isolate a full-length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a cDNA library, such as a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to isolate genomic clones via the screening of a genomic library.

[0065] PCR technology may also be utilized to isolate full-length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source (i.e., one known, or suspected, to express the IRF3 gene, such as, for example, B cells). A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see e.g., Sambrook et al., 1989, infra.

[0066] Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express an IRF3 polypeptide. According to this strategy, polypeptides expressed by the cloned cDNA are screened using standard antibody screening techniques in conjunction with antibodies raised against the IRF3 polypeptides of the invention. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) Additionally, screening can be accomplished by screening with labeled IRF3 proteins or fusion proteins, such as, for example, those described herein. Library clones detected via their reaction with such

labeled compounds can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

Vectors and Host Cells

[0067] The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors and/or nucleic acids of the invention and the production of IRF3 polypeptides or fragments thereof by recombinant techniques.

[0068] Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the present invention. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

[0069] In accordance with the present invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

[0070] Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

[0071] The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

[0072] The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable

promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0073] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells, such as *Saccharomyces* or *Pichia*; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0074] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. The availability of drugs which inhibit the function of the enzymes encoded by these selectable markers allows for selection of cell lines in which the vector sequences have been amplified after integration into the host cell's DNA. An advantage of glutamine synthase based vectors are the availabilty of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers including, for example, Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson

Biotechnol. Prog. 11:1 (1995) which are herein incorporated by reference.

[0075] Among vectors preferred for use in bacteria include pHE4-5 (ATCC Accession No. 209311; and variations thereof), pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to, pYES2, pY01, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and pA0815 (all available from Invitrogen, Carlsbad, CA). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

[0076] In one embodiment, the yeast *Pichia pastoris* is used to express IRF3 protein in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. *See*, Ellis, S.B., *et al., Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J, *et al., Yeast* 5:167-77 (1989); Tschopp, J.F., *et al., Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, an IRF3 polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0077] In one example, the plasmid vector pPIC9K is used to express DNA encoding an IRF3 polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R.

Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of an IRF3 protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

[0078] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0079] In one embodiment, high-level expression of a heterologous coding sequence, such as, for example, an IRF3 polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0080] The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may modulate the expression of the inserted gene sequences, or modify and process the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[0081] Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated

transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods In Molecular Biology* (1986).

[0082] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., IRF3 coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with IRF3 polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous IRF3 polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous IRF3 polynucleotide sequences via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al., Proc. Natl. Acad. Sci. USA 86*:8932-8935 (1989); and Zijlstra *et al., Nature 342*:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0083] The IRF3 polypeptide may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. For example, in one embodiment, polynucleotides encoding IRF3 polypeptides of the invention may be fused to the pelB pectate lyase signal sequence to increase the efficiency to expression and purification of such polypeptides in Gram-negative bacteria. See, US Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0084] A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al., Journal of Molecular Recognition* 8:52-58 (1995) and K. Johanson e*t al., The Journal of Biological Chemistry* 270:16:9459-9471 (1995).

[0085] Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0086] In addition, proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y. (1983), and Hunkapiller, *et al., Nature 310*:105-111 (1984)). For example, a polypeptide corresponding to a fragment of the IRF3 polypeptides of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the IRF3 polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino

butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0087] The invention additionally, encompasses IRF3 polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin; etc.

[0088] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0089] Also provided by the invention are chemically modified derivatives of IRF3 which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0090] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1

kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0091] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al., Appl. Biochem. Biotechnol. 56*:59-72 (1996); Vorobjev *et al., Nucleosides Nucleotides 18*:2745-2750 (1999); and Caliceti *et al., Bioconjug. Chem. 10*:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0092] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al., Exp. Hematol. 20*:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0093] As suggested above, polyethylene glycol may be attached to proteins via

linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0094] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0095] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992); Francis *et al., Intern. J. of Hematol. 68*:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0096] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is

directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

[0097] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0098] The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992).

[0099] As mentioned, the IRF3 proteins of the invention may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given IRF3 polypeptide. IRF3 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic IRF3 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme

moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al., Meth Enzymol 182*:626-646 (1990); Rattan *et al., Ann NY Acad Sci 663*:48-62 (1992)).

[0100] The IRF3 polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

[0101] IRF3 transcription factor polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of IRF3. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to inhibit proliferation of B cells, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of IRF3 by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

IRF3 Transgenics and "knock-outs"

[0102] The IRF3 proteins of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[0103] Any technique known in the art may be used to introduce the transgene (i.e., nucleic acids of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson *et al., Appl. Microbiol. Biotechnol. 40*:691-698 (1994); Carver *et al., Biotechnology (NY) 11*:1263-1270 (1993); Wright *et al., Biotechnology (NY) 9*:830-834 (1991); and Hoppe et al., US Patent Number 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten *et al., Proc. Natl. Acad. Sci., USA 82*:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson *et al., Cell 56*:313-321 (1989)); electroporation of cells or embryos (Lo, *Mol Cell. Biol. 3*:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer *et al., Science 259*:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano *et al., Cell 57*:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," *Intl. Rev. Cytol. 115*:171-229 (1989), which is incorporated by reference herein in its entirety. Further, the contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety. Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety. See also, U.S. Patent No. 5,464,764 (Capecchi, et al., Positive-Negative Selection Methods and Vectors); U.S. Patent No. 5,631,153 (Capecchi, et al., Cells and Non-Human Organisms Containing Predetermined Genomic Modifications and Positive-Negative Selection Methods and Vectors for Making Same); U.S. Patent No. 4,736,866 (Leder, et al., Transgenic Non-Human Animals); and U.S. Patent No. 4,873,191 (Wagner, et al., Genetic Transformation of Zygotes); each of which is hereby incorporated by reference in its entirety.

[0104] Any technique known in the art may be used to produce transgenic clones

containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell *et al., Nature 380*:64-66 (1996); Wilmut *et al., Nature 385*:810-813 (1997)), each of which is herein incorporated by reference in its entirety).

[0105] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric animals. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.* (*Proc. Natl. Acad. Sci. USA 89*:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu *et al.* (*Science 265*:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0106] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated

immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[0107] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

[0108] Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of IRF3 polypeptides, studying conditions and/or disorders associated with aberrant IRF3 expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

[0109] In further embodiments of the invention, cells that are genetically engineered to express the proteins of the invention, or alternatively, that are genetically engineered not to express the proteins of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells, etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or

promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. US Patent Number 5,399,349; and Mulligan & Wilson, US Patent Number 5,460,959, each of which is incorporated by reference herein in its entirety).

[0110] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

<u>IRF3 Polypeptides and Fragments</u>

[0111] The IRF3 proteins (polypeptides) of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to monomers and multimers of the IRF3 proteins (polypeptides) of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0112] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only IRF3 proteins of the invention (including IRF3 fragments, variants, and fusion proteins, as described herein). These homomers may contain IRF3 proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer of the invention is a multimer containing only IRF3 proteins having an identical polypeptide sequence. In another specific embodiment, a homomer of the invention is a multimer containing IRF3 proteins having different polypeptide sequences (e.g., IRF3 mutations containing proteins have

polypetide sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing IRF3 proteins having identical or different polypeptide sequences) or a homotrimer (e.g., containing IRF3 proteins having identical or different polypeptide sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0113] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to polypeptide sequences encoded by the IRF3 gene) in addition to the IRF3 proteins of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

[0114] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when proteins of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when proteins of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the IRF3 proteins of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence shown in Figure 1 (SEQ ID NO:2) or a polypeptide encoded by one of the deposited cDNA clones). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in an IRF3 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between

the heterologous sequence contained in an IRF3-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequences from a TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more IRF3 polypeptides of the invention are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple IRF3 polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0115] Another method for preparing multimer IRF3 polypeptides of the invention involves use of IRF3 polypeptides fused to a leucine zipper or isoleucine polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric IRF3 proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble IRF3 polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric IRF3 is recovered from the culture supernatant using techniques known in the art.

[0116] Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally ocurring trimeric proteins may be employed in preparing trimeric IRF3.

[0117] In another example, proteins of the invention are associated by interactions between

[0118] Flag® polypeptide sequence contained in Flag®-IRF3 fusion proteins of the

invention. In a further embodiment, associated proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag®-IRF3 fusion proteins of the invention and anti-Flag® antibody.

[0119] The multimers of the invention may be generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, proteins of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0120] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate

recombinant polypeptides of the invention which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0121] The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the IRF3 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

[0122] Accordingly, in one embodiment, the invention provides an isolated IRF3 polypeptide having the amino acid sequence encoded by the amino acid sequence in Figure 1 (SEQ ID NO:2), or a polypeptide comprising, or alternatively consisting of, a portion of the above polypeptides, such as for example, the IRF3 DNA binding domain (amino acids 1 to 107 of Figure 1 (SEQ ID NO:2)); the IRF3 nuclear export signal (amino acids 141-147 of Figure 1 (SEQ ID NO:2)); the IRF3 interferon regulatory factor association domain (amino acids 198 to 381 of Figure 1 (SEQ ID NO:2)); the IRF3 phosphorylation domain (amino acids 198 to 381 of Figure 1 (SEQ ID NO:2)); the IRF3 autoinhibitory domain (amino acids 198 to 381 of Figure 1 (SEQ ID NO:2)); the IRF3 polypeptide lacking the autoimhibitory domain (e.g., amino acids 1 to 407 of Figure 1 (SEQ ID NO:2)); and/or the IRF3 IRF7 interaction domain (amino acids 306 to 427 of Figure 1 (SEQ ID NO:2)).

[0123] Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of: an amino acid sequence contained in Figure 1 (SEQ ID NO:2); and encoded by a nucleic acid which hybridizes (e.g., under stringent hybridization conditions) to the complementary strand of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), or a fragment thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0124] Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single

continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of about amino acid residues: 4-8, 28-33, 66-71, 94-105, 118-128, 132-136, 153-157, 165-168, 173-178, 186-193, 198-202, 233-238, 304-316, 334-340, and 423-427, of SEQ ID NO:2 or Figure 1. In this context "about" includes the particularly recited ranges, an ranges larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, 300, 350, or 400 amino acids in length. Polynucleotides encoding these polypeptides are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0125] In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively consist of, one or more IRF3 domains. Preferred polypeptide fragments of the present invention include one, two, three or more members selected from the group: (a) a polypeptide comprising or alternatively, consisting of, the IRF3 DNA binding domain (amino acid residues 1 to 107 of Figure 1 (SEQ ID NO:2)); (b) a polypeptide comprising or alternatively, consisting of, the IRF3 nuclear export signal (amino acid residues 141 to 147 of Figure 1 (SEQ ID NO:2)); (c) a polypeptide comprising or alternatively, consisting of, the IRF3 interferon regulatory association domain (amino acid residues 198 to 381 Figure 1 (SEQ ID NO:2)); (d) a polypeptide comprising or alternatively, consisting of, the IRF3 phosphorylation domain (amino acid residues 382 to 407 of Figure 1 (SEQ ID NO:2)); (e) a polypeptide comprising, or alternatively, consisting of, one, two, three, four or more, epitope bearing portions of the IRF3 protein; or (f) any combination of polypeptides (a)-(e). Other preferred embodiments include IRF3 polypetides lacking the autoinhibitory domain (i.e. amino acids 1-407 of SEQ ID NO:2) and IRF3 poilypetides lacking the nuclear export signal (i.e., amino acid residues 1-140 of SEQ ID NO:2 fused to amino acid residues 148-427 of SEQ ID NO:2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0126] Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of IRF3. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions

("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) IRF3 (Figure 1 (SEQ ID NO:2)). Certain preferred regions are those set out in Figure 2 and Table 1 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in Figure 1 (SEQ ID NO:2), such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic; Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0127] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, antigenicity, ability to bind ISRE or PRDI-PRDIII containing promoters (e.g., ISG15 promoter or the IFN-alpha or IFN-beta promoters)) may still be retained. For instance, Ron et al., J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. The ability of shortened IRF3 "muteins" to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. As used herein, a "mutein" is a mutant protein including single or multiple amino acid substitutions, deletions, or additions (including fusion proteins). Whether a particular polypeptide lacking N-terminal residues of a complete full-length polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an IRF3 mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six IRF3 amino acid

residues may often evoke an immune response.

[0128] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the IRF3 amino acid sequence shown in Figure 1, up to the glutamine residue at position number 422 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues n^1-427 of Figure 1, where n^1 is an integer from 2 to 422 corresponding to the position of the amino acid residue in Figure 1 (SEQ ID NO:2).

[0129] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: G-2 to S-427; T-3 to S-427; P-4 to S-427; K-5 to S-427; P-6 to S-427; R-7 to S-427; X-8 to S-427; L-9 to S-427; P-10 to S-427; W-11 to S-427; L-12 to S-427; V-13 to S-427; S-14 to S-427; Q-15 to S-427; L-16 to S-427; D-17 to S-427; L-18 to S-427; G-19 to S-427; Q-20 to S-427; L-21 to S-427; E-22 to S-427; G-23 to S-427; V-24 to S-427; A-25 to S-427; W-26 to S-427; V-27 to S-427; N-28 to S-427; K-29 to S-427; S-30 to S-427; R-31 to S-427; T-32 to S-427; R-33 to S-427; F-34 to S-427; R-35 to S-427; I-36 to S-427; P-37 to S-427; W-38 to S-427; K-39 to S-427; H-40 to S-427; G-41 to S-427; L-42 to S-427; R-43 to S-427; Q-44 to S-427; D-45 to S-427; A-46 to S-427; Q-47 to S-427; Q-48 to S-427; E-49 to S-427; D-50 to S-427; F-51 to S-427; G-52 to S-427; I-53 to S-427; F-54 to S-427; Q-55 to S-427; A-56 to S-427; W-57 to S-427; A-58 to S-427; E-59 to S-427; A-60 to S-427; T-61 to S-427; G-62 to S-427; A-63 to S-427; Y-64 to S-427; V-65 to S-427; P-66 to S-427; G-67 to S-427; R-68 to S-427; D-69 to S-427; K-70 to S-427; P-71 to S-427; D-72 to S-427; L-73 to S-427; P-74 to S-427; T-75 to S-427; W-76 to S-427; K-77 to S-427; R-78 to S-427; N-79 to S-427; F-80 to S-427; R-81 to S-427; S-82 to S-427; A-83 to S-427; L-84 to S-427; N-85 to S-427; R-86 to S-427; K-87 to S-427; E-88 to S-427; G-89 to S-427; L-90 to S-427; R-91 to S-427; L-92 to S-427; A-93 to S-427; E-94 to S-427; D-95 to S-427; R-96 to S-427; S-97 to S-427; K-98 to S-427; D-99 to S-427; P-100 to S-427; H-101 to S-427; D-102 to S-427; P-103 to S-427; H-104 to S-427; K-105 to S-427; I-106 to S-427; Y-107 to S-427; E-108 to S-427; F-109 to S-427; V-110 to S-427; N-111 to S-427; S-112 to S-427; G-113 to S-427; V-114 to S-427; G-115 to S-427; D-116 to S-427; F-117 to S-427; S-118 to S-427; Q-119 to S-427; P-120 to S-427; D-121 to S-427; T-122 to S-427; S-123 to S-427; P-124 to S-427; D-125

to S-427; T-126 to S-427; N-127 to S-427; G-128 to S-427; G-129 to S-427; G-130 to S-427; S-131 to S-427; T-132 to S-427; S-133 to S-427; D-134 to S-427; T-135 to S-427; Q-136 to S-427; E-137 to S-427; D-138 to S-427; I-139 to S-427; L-140 to S-427; D-141 to S-427; E-142 to S-427; L-143 to S-427; L-144 to S-427; G-145 to S-427; N-146 to S-427; M-147 to S-427; V-148 to S-427; L-149 to S-427; A-150 to S-427; P-151 to S-427; L-152 to S-427; P-153 to S-427; D-154 to S-427; P-155 to S-427; G-156 to S-427; P-157 to S-427; P-158 to S-427; S-159 to S-427; L-160 to S-427; A-161 to S-427; V-162 to S-427; A-163 to S-427; P-164 to S-427; E-165 to S-427; P-166 to S-427; C-167 to S-427; P-168 to S-427; Q-169 to S-427; P-170 to S-427; L-171 to S-427; R-172 to S-427; S-173 to S-427; P-174 to S-427; S-175 to S-427; L-176 to S-427; D-177 to S-427; N-178 to S-427; P-179 to S-427; T-180 to S-427; P-181 to S-427; F-182 to S-427; P-183 to S-427; N-184 to S-427; L-185 to S-427; G-186 to S-427; P-187 to S-427; S-188 to S-427; E-189 to S-427; N-190 to S-427; P-191 to S-427; L-192 to S-427; K-193 to S-427; R-194 to S-427; L-195 to S-427; L-196 to S-427; V-197 to S-427; P-198 to S-427; G-199 to S-427; E-200 to S-427; E-201 to S-427; W-202 to S-427; E-203 to S-427; F-204 to S-427; E-205 to S-427; V-206 to S-427; T-207 to S-427; A-208 to S-427; F-209 to S-427; Y-210 to S-427; R-211 to S-427; G-212 to S-427; R-213 to S-427; Q-214 to S-427; V-215 to S-427; F-216 to S-427; Q-217 to S-427; Q-218 to S-427; T-219 to S-427; I-220 to S-427; S-221 to S-427; C-222 to S-427; P-223 to S-427; E-224 to S-427; G-225 to S-427; L-226 to S-427; R-227 to S-427; L-228 to S-427; V-229 to S-427; G-230 to S-427; S-231 to S-427; E-232 to S-427; V-233 to S-427; G-234 to S-427; D-235 to S-427; R-236 to S-427; T-237 to S-427; L-238 to S-427; P-239 to S-427; G-240 to S-427; W-241 to S-427; P-242 to S-427; V-243 to S-427; T-244 to S-427; L-245 to S-427; P-246 to S-427; D-247 to S-427; P-248 to S-427; G-249 to S-427; M-250 to S-427; S-251 to S-427; L-252 to S-427; T-253 to S-427; D-254 to S-427; R-255 to S-427; G-256 to S-427; V-257 to S-427; M-258 to S-427; S-259 to S-427; Y-260 to S-427; V-261 to S-427; R-262 to S-427; H-263 to S-427; V-264 to S-427; L-265 to S-427; S-266 to S-427; C-267 to S-427; L-268 to S-427; G-269 to S-427; G-270 to S-427; G-271 to S-427; L-272 to S-427; A-273 to S-427; L-274 to S-427; W-275 to S-427; R-276 to S-427; A-277 to S-427; G-278 to S-427; Q-279 to S-427; W-280 to S-427; L-281 to S-427; W-282 to S-427; A-283 to S-427; Q-284 to S-427; R-285 to S-427; L-286 to S-427; G-287 to S-427; H-288 to S-427; C-289 to S-427; H-290 to S-427; T-291 to S-427; Y-292 to S-427; W-293 to S-427; A-294 to S-427;

V-295 to S-427; S-296 to S-427; E-297 to S-427; E-298 to S-427; L-299 to S-427; L-300 to S-427; P-301 to S-427; N-302 to S-427; S-303 to S-427; G-304 to S-427; H-305 to S-427; G-306 to S-427; P-307 to S-427; D-308 to S-427; G-309 to S-427; E-310 to S-427; V-311 to S-427; P-312 to S-427; K-313 to S-427; D-314 to S-427; K-315 to S-427; E-316 to S-427; G-317 to S-427; G-318 to S-427; V-319 to S-427; F-320 to S-427; D-321 to S-427; L-322 to S-427; G-323 to S-427; P-324 to S-427; F-325 to S-427; I-326 to S-427; V-327 to S-427; D-328 to S-427; L-329 to S-427; I-330 to S-427; T-331 to S-427; F-332 to S-427; T-333 to S-427; E-334 to S-427; G-335 to S-427; S-336 to S-427; G-337 to S-427; R-338 to S-427; S-339 to S-427; P-340 to S-427; R-341 to S-427; Y-342 to S-427; A-343 to S-427; L-344 to S-427; W-345 to S-427; F-346 to S-427; C-347 to S-427; V-348 to S-427; G-349 to S-427; E-350 to S-427; S-351 to S-427; W-352 to S-427; P-353 to S-427; Q-354 to S-427; D-355 to S-427; Q-356 to S-427; P-357 to S-427; W-358 to S-427; T-359 to S-427; K-360 to S-427; R-361 to S-427; L-362 to S-427; V-363 to S-427; M-364 to S-427; V-365 to S-427; K-366 to S-427; V-367 to S-427; V-368 to S-427; P-369 to S-427; T-370 to S-427; C-371 to S-427; L-372 to S-427; R-373 to S-427; A-374 to S-427; L-375 to S-427; V-376 to S-427; E-377 to S-427; M-378 to S-427; A-379 to S-427; R-380 to S-427; V-381 to S-427; G-382 to S-427; G-383 to S-427; A-384 to S-427; S-385 to S-427; S-386 to S-427; L-387 to S-427; E-388 to S-427; N-389 to S-427; T-390 to S-427; V-391 to S-427; D-392 to S-427; L-393 to S-427; H-394 to S-427; I-395 to S-427; S-396 to S-427; N-397 to S-427; S-398 to S-427; H-399 to S-427; P-400 to S-427; L-401 to S-427; S-402 to S-427; L-403 to S-427; T-404 to S-427; S-405 to S-427; D-406 to S-427; Q-407 to S-427; Y-408 to S-427; K-409 to S-427; A-410 to S-427; Y-411 to S-427; L-412 to S-427; Q-413 to S-427; D-414 to S-427; L-415 to S-427; V-416 to S-427; E-417 to S-427; G-418 to S-427; M-419 to S-427; D-420 to S-427; F-421 to S-427; and/or Q-422 to S-427 of the IRF3 sequence shown in Figure 1. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0130] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, antigenicity, ability to bind ISRE or PRDI-PRDIII containing promoters (e.g., ISG15 promoter or the IFN-alpha or IFN-beta promoters)) may still be retained. For example the ability of the shortened IRF3 mutein to induce and/or bind to antibodies which recognize the complete IRF3

polypeptide generally will be retained when less than the majority of the residues of the complete polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an IRF3 mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six IRF3 amino acid residues may often evoke an immune response.

[0131] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the IRF3 polypeptide shown in Figure 1, up to the arginine residue at position number 7, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m^1 of Figure 1, where m^1 is an integer from 7 to 426 corresponding to the position of the amino acid residue in Figure 1 (SEQ ID NO:2).

[0132] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: M-1 to E-426; M-1 to G-425; M-1 to P-424; M-1 to G-423; M-1 to Q-422; M-1 to F-421; M-1 to D-420; M-1 to M-419; M-1 to G-418; M-1 to E-417; M-1 to V-416; M-1 to L-415; M-1 to D-414; M-1 to Q-413; M-1 to L-412; M-1 to Y-411; M-1 to A-410; M-1 to K-409; M-1 to Y-408; M-1 to Q-407; M-1 to D-406; M-1 to S-405; M-1 to T-404; M-1 to L-403; M-1 to S-402; M-1 to L-401; M-1 to P-400; M-1 to H-399; M-1 to S-398; M-1 to N-397; M-1 to S-396; M-1 to I-395; M-1 to H-394; M-1 to L-393; M-1 to D-392; M-1 to V-391; M-1 to T-390; M-1 to N-389; M-1 to E-388; M-1 to L-387; M-1 to S-386; M-1 to S-385; M-1 to A-384; M-1 to G-383; M-1 to G-382; M-1 to V-381; M-1 to R-380; M-1 to A-379; M-1 to M-378; M-1 to E-377; M-1 to V-376; M-1 to L-375; M-1 to A-374; M-1 to R-373; M-1 to L-372; M-1 to C-371; M-1 to T-370; M-1 to P-369; M-1 to V-368; M-1 to V-367; M-1 to K-366; M-1 to V-365; M-1 to M-364; M-1 to V-363; M-1 to L-362; M-1 to R-361; M-1 to K-360; M-1 to T-359; M-1 to W-358; M-1 to P-357; M-1 to Q-356; M-1 to D-355; M-1 to Q-354; M-1 to P-353; M-1 to W-352; M-1 to S-351; M-1 to E-350; M-1 to G-349; M-1 to V-348; M-1 to C-347; M-1 to F-346; M-1 to W-345; M-1 to L-344; M-1 to A-343; M-1 to Y-342; M-1 to R-341; M-1 to P-340; M-1 to S-339; M-1 to R-338; M-1

to G-337; M-1 to S-336; M-1 to G-335; M-1 to E-334; M-1 to T-333; M-1 to F-332; M-1 to T-331; M-1 to I-330; M-1 to L-329; M-1 to D-328; M-1 to V-327; M-1 to I-326; M-1 to F-325; M-1 to P-324; M-1 to G-323; M-1 to L-322; M-1 to D-321; M-1 to F-320; M-1 to V-319; M-1 to G-318; M-1 to G-317; M-1 to E-316; M-1 to K-315; M-1 to D-314; M-1 to K-313; M-1 to P-312; M-1 to V-311; M-1 to E-310; M-1 to G-309; M-1 to D-308; M-1 to P-307; M-1 to G-306; M-1 to H-305; M-1 to G-304; M-1 to S-303; M-1 to N-302; M-1 to P-301; M-1 to L-300; M-1 to L-299; M-1 to E-298; M-1 to E-297; M-1 to S-296; M-1 to V-295; M-1 to A-294; M-1 to W-293; M-1 to Y-292; M-1 to T-291; M-1 to H-290; M-1 to C-289; M-1 to H-288; M-1 to G-287; M-1 to L-286; M-1 to R-285; M-1 to Q-284; M-1 to A-283; M-1 to W-282; M-1 to L-281; M-1 to W-280; M-1 to Q-279; M-1 to G-278; M-1 to A-277; M-1 to R-276; M-1 to W-275; M-1 to L-274; M-1 to A-273; M-1 to L-272; M-1 to G-271; M-1 to G-270; M-1 to G-269; M-1 to L-268; M-1 to C-267; M-1 to S-266; M-1 to L-265; M-1 to V-264; M-1 to H-263; M-1 to R-262; M-1 to V-261; M-1 to Y-260; M-1 to S-259; M-1 to M-258; M-1 to V-257; M-1 to G-256; M-1 to R-255; M-1 to D-254; M-1 to T-253; M-1 to L-252; M-1 to S-251; M-1 to M-250; M-1 to G-249; M-1 to P-248; M-1 to D-247; M-1 to P-246; M-1 to L-245; M-1 to T-244; M-1 to V-243; M-1 to P-242; M-1 to W-241; M-1 to G-240; M-1 to P-239; M-1 to L-238; M-1 to T-237; M-1 to R-236; M-1 to D-235; M-1 to G-234; M-1 to V-233; M-1 to E-232; M-1 to S-231; M-1 to G-230; M-1 to V-229; M-1 to L-228; M-1 to R-227; M-1 to L-226; M-1 to G-225; M-1 to E-224; M-1 to P-223; M-1 to C-222; M-1 to S-221; M-1 to I-220; M-1 to T-219; M-1 to Q-218; M-1 to Q-217; M-1 to F-216; M-1 to V-215; M-1 to Q-214; M-1 to R-213; M-1 to G-212; M-1 to R-211; M-1 to Y-210; M-1 to F-209; M-1 to A-208; M-1 to T-207; M-1 to V-206; M-1 to E-205; M-1 to F-204; M-1 to E-203; M-1 to W-202; M-1 to E-201; M-1 to E-200; M-1 to G-199; M-1 to P-198; M-1 to V-197; M-1 to L-196; M-1 to L-195; M-1 to R-194; M-1 to K-193; M-1 to L-192; M-1 to P-191; M-1 to N-190; M-1 to E-189; M-1 to S-188; M-1 to P-187; M-1 to G-186; M-1 to L-185; M-1 to N-184; M-1 to P-183; M-1 to F-182; M-1 to P-181; M-1 to T-180; M-1 to P-179; M-1 to N-178; M-1 to D-177; M-1 to L-176; M-1 to S-175; M-1 to P-174; M-1 to S-173; M-1 to R-172; M-1 to L-171; M-1 to P-170; M-1 to Q-169; M-1 to P-168; M-1 to C-167; M-1 to P-166; M-1 to E-165; M-1 to P-164; M-1 to A-163; M-1 to V-162; M-1 to A-161; M-1 to L-160; M-1 to S-159; M-1 to P-158; M-1 to P-157; M-1 to G-156; M-1 to P-155; M-1 to D-154; M-1 to P-153; M-1 to L-152; M-1 to P-151; M-1 to A-150; M-1 to L-149; M-1 to V-148; M-1 to M-147; M-1 to N-146; M-1

to G-145; M-1 to L-144; M-1 to L-143; M-1 to E-142; M-1 to D-141; M-1 to L-140; M-1 to I-139; M-1 to D-138; M-1 to E-137; M-1 to Q-136; M-1 to T-135; M-1 to D-134; M-1 to S-133; M-1 to T-132; M-1 to S-131; M-1 to G-130; M-1 to G-129; M-1 to G-128; M-1 to N-127; M-1 to T-126; M-1 to D-125; M-1 to P-124; M-1 to S-123; M-1 to T-122; M-1 to D-121; M-1 to P-120; M-1 to Q-119; M-1 to S-118; M-1 to F-117; M-1 to D-116; M-1 to G-115; M-1 to V-114; M-1 to G-113; M-1 to S-112; M-1 to N-111; M-1 to V-110; M-1 to F-109; M-1 to E-108; M-1 to Y-107; M-1 to I-106; M-1 to K-105; M-1 to H-104; M-1 to P-103; M-1 to D-102; M-1 to H-101; M-1 to P-100; M-1 to D-99; M-1 to K-98; M-1 to S-97; M-1 to R-96; M-1 to D-95; M-1 to E-94; M-1 to A-93; M-1 to L-92; M-1 to R-91; M-1 to L-90; M-1 to G-89; M-1 to E-88; M-1 to K-87; M-1 to R-86; M-1 to N-85; M-1 to L-84; M-1 to A-83; M-1 to S-82; M-1 to R-81; M-1 to F-80; M-1 to N-79; M-1 to R-78; M-1 to K-77; M-1 to W-76; M-1 to T-75; M-1 to P-74; M-1 to L-73; M-1 to D-72; M-1 to P-71; M-1 to K-70; M-1 to D-69; M-1 to R-68; M-1 to G-67; M-1 to P-66; M-1 to V-65; M-1 to Y-64; M-1 to A-63; M-1 to G-62; M-1 to T-61; M-1 to A-60; M-1 to E-59; M-1 to A-58; M-1 to W-57; M-1 to A-56; M-1 to Q-55; M-1 to F-54; M-1 to I-53; M-1 to G-52; M-1 to F-51; M-1 to D-50; M-1 to E-49; M-1 to Q-48; M-1 to Q-47; M-1 to A-46; M-1 to D-45; M-1 to Q-44; M-1 to R-43; M-1 to L-42; M-1 to G-41; M-1 to H-40; M-1 to K-39; M-1 to W-38; M-1 to P-37; M-1 to I-36; M-1 to R-35; M-1 to F-34; M-1 to R-33; M-1 to T-32; M-1 to R-31; M-1 to S-30; M-1 to K-29; M-1 to N-28; M-1 to V-27; M-1 to W-26; M-1 to A-25; M-1 to V-24; M-1 to G-23; M-1 to E-22; M-1 to L-21; M-1 to Q-20; M-1 to G-19; M-1 to L-18; M-1 to D-17; M-1 to L-16; M-1 to Q-15; M-1 to S-14; M-1 to V-13; M-1 to L-12; M-1 to W-11; M-1 to P-10; M-1 to L-9; M-1 to X-8; and/or M-1 to R-7 of the IRF3 sequence shown in Figure 1. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0133] The invention also provides polynucleotides encoding polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues n^1- m^1 and/or n^2- m^1 of Figure 1 (i.e., SEQ ID NO:2), where n^1, n^2, and m^1 are integers as described above. Thus, any of the above listed N- or C-terminal deletions can be combined to produce a polynucleotide encoding an N- and C-terminal deleted IRF3 polypeptide.

[0134] The present invention encompasses IRF3 polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of

Figure 1 (SEQ ID NO:2), or an epitope of a polypeptide sequence encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:1 (e.g., under stringent hybridization conditions or lower stringency hybridization conditions as defined herein). The present invention further encompasses polynucleotide sequences encoding an epitope of an IRF3 polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:2), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand (e.g., under stringent hybridization conditions or lower stringency hybridization conditions defined herein).

[0135] The term “epitopes,” as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An “immunogenic epitope,” as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described herein. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods. Antibodies that specifically bind IRF3 are also encompassed by the invention.

[0136] The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

[0137] Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

[0138] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length.

[0139] Non-limiting examples of antigenic polypeptides of the invention include one, two, three, four, five, or more members selected from the group: a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-4 to about Xaa 8 in Figures 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Asn-28 to about Arg-33 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-66 to about Pro-71 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Glu-94 to Lys 105 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser 118 to about Gly-128 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Thr-132 to Gln-136 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser-118 to about Gly-128 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-153 to about Pro-157 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Glu-165 to about Pro-168 in

Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser-173 to about Asn-178 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Gly-186 to about Lys-193 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-198 to about Trp-202 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Val-233 to about Leu-238 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Gly-304 to about Glu-316 in Figure 1 (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Glu-334 to about Pro-340 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about Gly-423 to about Ser-427 in Figure 1 (SEQ ID NO:2);. In this context, "about" means the particularly recited ranges and ranges that are larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini. These polypeptide fragments have been determined to bear antigenic epitopes of the IRF3 polypeptide by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 1 and Table I, above. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)). Polynucleotides encoding these polypeptides are encompassed by the invention. Additionally, antibodies that bind to one or more of these polypeptides are also encompassed by the invention.

[0140] Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more

immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0141] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0142] As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including, but not limited to, recombinant human albumin and

fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, EP Patent 0413622, and U.S. Patent No. 5,766,883, herein incorporated by reference in their entirety). Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA 88:8972-8976(1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

[0143] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-

76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of IRF3 polynucleotides corresponding to Figure 1 (SEQ ID NO:1) and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0144] It will be recognized in the art that some amino acid sequences of IRF3 can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the IRF3 transcription factor, which show substantial IRF3 transcription factor activity or which include regions of IRF3 proteins, such as the protein portions discussed herein. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in J.U. Bowie *et al., Science 247*:1306-1310 (1990).

[0145] Thus, the fragment, derivative, or analog of the polypeptide of Figure 1 (SEQ ID NO:2), may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the

mature polypeptide, such as an IgG Fc region, or human serum albumin or fragments or variants thereof, or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

[0146] Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the IRF3 transcription factor protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol.* *2*:331-340 (1967); Robbins *et al., Diabetes* *36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems* *10*:307-377 (1993)).

[0147] The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature* *361*:266-268 (1993), describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the IRF3 polypeptide receptors of the present invention may include one or more amino acid substitutions, deletions, or additions, either from natural mutations or human manipulation.

[0148] As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table II).

TABLE II. Conservative Amino Acid Substitutions

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0149] In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of Figure 1 and/or any of the polypeptide fragments described herein (e.g., the DNA binding domain, nuclear export signal, interferon regulatory factor association domain, phosphorylation domain, or the autoinhibitory domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2.

[0150] In another embodiment, site directed changes at the amino acid level of IRF3 can be made by replacing a particular amino acid with a conservative substitution. Preferred conservative substitution mutations of the IRF3 amino acid sequence provided in SEQ ID NO:2 include: M1 replaced with A, G, I, L, S, T, or V; G2 replaced with A, I, L, S, T, M, or V; T3 replaced with A, G, I, L, S, M, or V; K5 replaced with H, or R; R7 replaced with H, or K; L9 replaced with A, G, I, S, T, M, or V; W11 replaced with F, or Y; L12 replaced with A, G, I, S, T, M, or V; V13 replaced with A, G, I, L, S, T, or M; S14 replaced with A, G, I, L, T, M, or V; Q15 replaced with N; L16 replaced with A, G, I, S, T, M, or V; D17 replaced with E; L18 replaced with A, G, I, S, T, M, or V; G19 replaced with A, I, L, S, T, M, or V; Q20 replaced with N; L21 replaced with A, G, I, S, T, M, or

V; E22 replaced with D; G23 replaced with A, I, L, S, T, M, or V; V24 replaced with A, G, I, L, S, T, or M; A25 replaced with G, I, L, S, T, M, or V; W26 replaced with F, or Y; V27 replaced with A, G, I, L, S, T, or M; N28 replaced with Q; K29 replaced with H, or R; S30 replaced with A, G, I, L, T, M, or V; R31 replaced with H, or K; T32 replaced with A, G, I, L, S, M, or V; R33 replaced with H, or K; F34 replaced with W, or Y; R35 replaced with H, or K; I36 replaced with A, G, L, S, T, M, or V; W38 replaced with F, or Y; K39 replaced with H, or R; H40 replaced with K, or R; G41 replaced with A, I, L, S, T, M, or V; L42 replaced with A, G, I, S, T, M, or V; R43 replaced with H, or K; Q44 replaced with N; D45 replaced with E; A46 replaced with G, I, L, S, T, M, or V; Q47 replaced with N; Q48 replaced with N; E49 replaced with D; D50 replaced with E; F51 replaced with W, or Y; G52 replaced with A, I, L, S, T, M, or V; I53 replaced with A, G, L, S, T, M, or V; F54 replaced with W, or Y; Q55 replaced with N; A56 replaced with G, I, L, S, T, M, or V; W57 replaced with F, or Y; A58 replaced with G, I, L, S, T, M, or V; E59 replaced with D; A60 replaced with G, I, L, S, T, M, or V; T61 replaced with A, G, I, L, S, M, or V; G62 replaced with A, I, L, S, T, M, or V; A63 replaced with G, I, L, S, T, M, or V; Y64 replaced with F, or W; V65 replaced with A, G, I, L, S, T, or M; G67 replaced with A, I, L, S, T, M, or V; R68 replaced with H, or K; D69 replaced with E; K70 replaced with H, or R; D72 replaced with E; L73 replaced with A, G, I, S, T, M, or V; T75 replaced with A, G, I, L, S, M, or V; W76 replaced with F, or Y; K77 replaced with H, or R; R78 replaced with H, or K; N79 replaced with Q; F80 replaced with W, or Y; R81 replaced with H, or K; S82 replaced with A, G, I, L, T, M, or V; A83 replaced with G, I, L, S, T, M, or V; L84 replaced with A, G, I, S, T, M, or V; N85 replaced with Q; R86 replaced with H, or K; K87 replaced with H, or R; E88 replaced with D; G89 replaced with A, I, L, S, T, M, or V; L90 replaced with A, G, I, S, T, M, or V; R91 replaced with H, or K; L92 replaced with A, G, I, S, T, M, or V; A93 replaced with G, I, L, S, T, M, or V; E94 replaced with D; D95 replaced with E; R96 replaced with H, or K; S97 replaced with A, G, I, L, T, M, or V; K98 replaced with H, or R; D99 replaced with E; H101 replaced with K, or R; D102 replaced with E; H104 replaced with K, or R; K105 replaced with H, or R; I106 replaced with A, G, L, S, T, M, or V; Y107 replaced with F, or W; E108 replaced with D; F109 replaced with W, or Y; V110 replaced with A, G, I, L, S, T, or M; N111 replaced with Q; S112 replaced with A, G, I, L, T, M, or V; G113 replaced with A, I, L, S, T, M, or V; V114 replaced with A, G, I, L, S, T, or M; G115 replaced with A, I, L,

S, T, M, or V; D116 replaced with E; F117 replaced with W, or Y; S118 replaced with A, G, I, L, T, M, or V; Q119 replaced with N; D121 replaced with E; T122 replaced with A, G, I, L, S, M, or V; S123 replaced with A, G, I, L, T, M, or V; D125 replaced with E; T126 replaced with A, G, I, L, S, M, or V; N127 replaced with Q; G128 replaced with A, I, L, S, T, M, or V; G129 replaced with A, I, L, S, T, M, or V; G130 replaced with A, I, L, S, T, M, or V; S131 replaced with A, G, I, L, T, M, or V; T132 replaced with A, G, I, L, S, M, or V; S133 replaced with A, G, I, L, T, M, or V; D134 replaced with E; T135 replaced with A, G, I, L, S, M, or V; Q136 replaced with N; E137 replaced with D; D138 replaced with E; I139 replaced with A, G, L, S, T, M, or V; L140 replaced with A, G, I, S, T, M, or V; D141 replaced with E; E142 replaced with D; L143 replaced with A, G, I, S, T, M, or V; L144 replaced with A, G, I, S, T, M, or V; G145 replaced with A, I, L, S, T, M, or V; N146 replaced with Q; M147 replaced with A, G, I, L, S, T, or V; V148 replaced with A, G, I, L, S, T, or M; L149 replaced with A, G, I, S, T, M, or V; A150 replaced with G, I, L, S, T, M, or V; L152 replaced with A, G, I, S, T, M, or V; D154 replaced with E; G156 replaced with A, I, L, S, T, M, or V; S159 replaced with A, G, I, L, T, M, or V; L160 replaced with A, G, I, S, T, M, or V; A161 replaced with G, I, L, S, T, M, or V; V162 replaced with A, G, I, L, S, T, or M; A163 replaced with G, I, L, S, T, M, or V; E165 replaced with D; Q169 replaced with N; L171 replaced with A, G, I, S, T, M, or V; R172 replaced with H, or K; S173 replaced with A, G, I, L, T, M, or V; S175 replaced with A, G, I, L, T, M, or V; L176 replaced with A, G, I, S, T, M, or V; D177 replaced with E; N178 replaced with Q; T180 replaced with A, G, I, L, S, M, or V; F182 replaced with W, or Y; N184 replaced with Q; L185 replaced with A, G, I, S, T, M, or V; G186 replaced with A, I, L, S, T, M, or V; S188 replaced with A, G, I, L, T, M, or V; E189 replaced with D; N190 replaced with Q; L192 replaced with A, G, I, S, T, M, or V; K193 replaced with H, or R; R194 replaced with H, or K; L195 replaced with A, G, I, S, T, M, or V; L196 replaced with A, G, I, S, T, M, or V; V197 replaced with A, G, I, L, S, T, or M; G199 replaced with A, I, L, S, T, M, or V; E200 replaced with D; E201 replaced with D; W202 replaced with F, or Y; E203 replaced with D; F204 replaced with W, or Y; E205 replaced with D; V206 replaced with A, G, I, L, S, T, or M; T207 replaced with A, G, I, L, S, M, or V; A208 replaced with G, I, L, S, T, M, or V; F209 replaced with W, or Y; Y210 replaced with F, or W; R211 replaced with H, or K; G212 replaced with A, I, L, S, T, M, or V; R213 replaced with H, or K; Q214 replaced with N; V215 replaced with A, G, I, L, S, T,

or M; F216 replaced with W, or Y; Q217 replaced with N; Q218 replaced with N; T219 replaced with A, G, I, L, S, M, or V; I220 replaced with A, G, L, S, T, M, or V; S221 replaced with A, G, I, L, T, M, or V; E224 replaced with D; G225 replaced with A, I, L, S, T, M, or V; L226 replaced with A, G, I, S, T, M, or V; R227 replaced with H, or K; L228 replaced with A, G, I, S, T, M, or V; V229 replaced with A, G, I, L, S, T, or M; G230 replaced with A, I, L, S, T, M, or V; S231 replaced with A, G, I, L, T, M, or V; E232 replaced with D; V233 replaced with A, G, I, L, S, T, or M; G234 replaced with A, I, L, S, T, M, or V; D235 replaced with E; R236 replaced with H, or K; T237 replaced with A, G, I, L, S, M, or V; L238 replaced with A, G, I, S, T, M, or V; G240 replaced with A, I, L, S, T, M, or V; W241 replaced with F, or Y; V243 replaced with A, G, I, L, S, T, or M; T244 replaced with A, G, I, L, S, M, or V; L245 replaced with A, G, I, S, T, M, or V; D247 replaced with E; G249 replaced with A, I, L, S, T, M, or V; M250 replaced with A, G, I, L, S, T, or V; S251 replaced with A, G, I, L, T, M, or V; L252 replaced with A, G, I, S, T, M, or V; T253 replaced with A, G, I, L, S, M, or V; D254 replaced with E; R255 replaced with H, or K; G256 replaced with A, I, L, S, T, M, or V; V257 replaced with A, G, I, L, S, T, or M; M258 replaced with A, G, I, L, S, T, or V; S259 replaced with A, G, I, L, T, M, or V; Y260 replaced with F, or W; V261 replaced with A, G, I, L, S, T, or M; R262 replaced with H, or K; H263 replaced with K, or R; V264 replaced with A, G, I, L, S, T, or M; L265 replaced with A, G, I, S, T, M, or V; S266 replaced with A, G, I, L, T, M, or V; L268 replaced with A, G, I, S, T, M, or V; G269 replaced with A, I, L, S, T, M, or V; G270 replaced with A, I, L, S, T, M, or V; G271 replaced with A, I, L, S, T, M, or V; L272 replaced with A, G, I, S, T, M, or V; A273 replaced with G, I, L, S, T, M, or V; L274 replaced with A, G, I, S, T, M, or V; W275 replaced with F, or Y; R276 replaced with H, or K; A277 replaced with G, I, L, S, T, M, or V; G278 replaced with A, I, L, S, T, M, or V; Q279 replaced with N; W280 replaced with F, or Y; L281 replaced with A, G, I, S, T, M, or V; W282 replaced with F, or Y; A283 replaced with G, I, L, S, T, M, or V; Q284 replaced with N; R285 replaced with H, or K; L286 replaced with A, G, I, S, T, M, or V; G287 replaced with A, I, L, S, T, M, or V; H288 replaced with K, or R; H290 replaced with K, or R; T291 replaced with A, G, I, L, S, M, or V; Y292 replaced with F, or W; W293 replaced with F, or Y; A294 replaced with G, I, L, S, T, M, or V; V295 replaced with A, G, I, L, S, T, or M; S296 replaced with A, G, I, L, T, M, or V; E297 replaced with D; E298 replaced with D; L299 replaced with A, G, I, S, T, M, or V; L300 replaced with

A, G, I, S, T, M, or V; N302 replaced with Q; S303 replaced with A, G, I, L, T, M, or V; G304 replaced with A, I, L, S, T, M, or V; H305 replaced with K, or R; G306 replaced with A, I, L, S, T, M, or V; D308 replaced with E; G309 replaced with A, I, L, S, T, M, or V; E310 replaced with D; V311 replaced with A, G, I, L, S, T, or M; K313 replaced with H, or R; D314 replaced with E; K315 replaced with H, or R; E316 replaced with D; G317 replaced with A, I, L, S, T, M, or V; G318 replaced with A, I, L, S, T, M, or V; V319 replaced with A, G, I, L, S, T, or M; F320 replaced with W, or Y; D321 replaced with E; L322 replaced with A, G, I, S, T, M, or V; G323 replaced with A, I, L, S, T, M, or V; F325 replaced with W, or Y; I326 replaced with A, G, L, S, T, M, or V; V327 replaced with A, G, I, L, S, T, or M; D328 replaced with E; L329 replaced with A, G, I, S, T, M, or V; I330 replaced with A, G, L, S, T, M, or V; T331 replaced with A, G, I, L, S, M, or V; F332 replaced with W, or Y; T333 replaced with A, G, I, L, S, M, or V; E334 replaced with D; G335 replaced with A, I, L, S, T, M, or V; S336 replaced with A, G, I, L, T, M, or V; G337 replaced with A, I, L, S, T, M, or V; R338 replaced with H, or K; S339 replaced with A, G, I, L, T, M, or V; R341 replaced with H, or K; Y342 replaced with F, or W; A343 replaced with G, I, L, S, T, M, or V; L344 replaced with A, G, I, S, T, M, or V; W345 replaced with F, or Y; F346 replaced with W, or Y; V348 replaced with A, G, I, L, S, T, or M; G349 replaced with A, I, L, S, T, M, or V; E350 replaced with D; S351 replaced with A, G, I, L, T, M, or V; W352 replaced with F, or Y; Q354 replaced with N; D355 replaced with E; Q356 replaced with N; W358 replaced with F, or Y; T359 replaced with A, G, I, L, S, M, or V; K360 replaced with H, or R; R361 replaced with H, or K; L362 replaced with A, G, I, S, T, M, or V; V363 replaced with A, G, I, L, S, T, or M; M364 replaced with A, G, I, L, S, T, or V; V365 replaced with A, G, I, L, S, T, or M; K366 replaced with H, or R; V367 replaced with A, G, I, L, S, T, or M; V368 replaced with A, G, I, L, S, T, or M; T370 replaced with A, G, I, L, S, M, or V; L372 replaced with A, G, I, S, T, M, or V; R373 replaced with H, or K; A374 replaced with G, I, L, S, T, M, or V; L375 replaced with A, G, I, S, T, M, or V; V376 replaced with A, G, I, L, S, T, or M; E377 replaced with D; M378 replaced with A, G, I, L, S, T, or V; A379 replaced with G, I, L, S, T, M, or V; R380 replaced with H, or K; V381 replaced with A, G, I, L, S, T, or M; G382 replaced with A, I, L, S, T, M, or V; G383 replaced with A, I, L, S, T, M, or V; A384 replaced with G, I, L, S, T, M, or V; S385 replaced with A, G, I, L, T, M, or V; S386 replaced with A, G, I, L, T, M, or V; L387 replaced with A, G, I, S, T, M, or V;

E388 replaced with D; N389 replaced with Q; T390 replaced with A, G, I, L, S, M, or V; V391 replaced with A, G, I, L, S, T, or M; D392 replaced with E; L393 replaced with A, G, I, S, T, M, or V; H394 replaced with K, or R; I395 replaced with A, G, L, S, T, M, or V; S396 replaced with A, G, I, L, T, M, or V; N397 replaced with Q; S398 replaced with A, G, I, L, T, M, or V; H399 replaced with K, or R; L401 replaced with A, G, I, S, T, M, or V; S402 replaced with A, G, I, L, T, M, or V; L403 replaced with A, G, I, S, T, M, or V; T404 replaced with A, G, I, L, S, M, or V; S405 replaced with A, G, I, L, T, M, or V; D406 replaced with E; Q407 replaced with N; Y408 replaced with F, or W; K409 replaced with H, or R; A410 replaced with G, I, L, S, T, M, or V; Y411 replaced with F, or W; L412 replaced with A, G, I, S, T, M, or V; Q413 replaced with N; D414 replaced with E; L415 replaced with A, G, I, S, T, M, or V; V416 replaced with A, G, I, L, S, T, or M; E417 replaced with D; G418 replaced with A, I, L, S, T, M, or V; M419 replaced with A, G, I, L, S, T, or V; D420 replaced with E; F421 replaced with W, or Y; Q422 replaced with N; G423 replaced with A, I, L, S, T, M, or V; G425 replaced with A, I, L, S, T, M, or V; E426 replaced with D; and/or S427 replaced with A, G, I, L, T, M, or V. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting IRF3 of the invention may be routinely screened for IRF3 functional activity and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility). Preferably, the resulting proteins of the invention have an increased and/or a decreased IRF3 functional activity. More preferably, the resulting IRF3 proteins of the invention have more than one increased and/or decreased IRF3 functional activity and/or physical property.

[0151] Amino acids in the IRF3 proteins of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as DNA binding or ability to stimulate transcription from promoters containing IRF3 binding elements (*e.g.*, ISRE elements, or PRDI-PRDIII elements). Sites that are critical for DNA binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

[0152] Of special interest are substitutions of charged amino acids with other charged or neutral amino acids that may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al., Clin. Exp. Immunol.* *2*:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland *et al., Crit. Rev. Therapeutic Drug Carrier Systems* *10*:307-377 (1993).

[0153] In another embodiment, the invention provides for polypeptides having amino acid sequences containing non-conservative substitutions of the amino acid sequence provided in SEQ ID NO:2. For example, non-conservative substitutions of the IRF3 protein sequence provided in SEQ ID NO:2 include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G2 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T3 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P4 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K5 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P6 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R7 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L9 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P10 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W11 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q15 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D17 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L18 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G19 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L21 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E22 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G23 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W26 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V27 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N28 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K29 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R31 replaced with D, E, A, G, I,

L, S, T, M, V, N, Q, F, W, Y, P, or C; T32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R33 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F34 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R35 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P37 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W38 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K39 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H40 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R43 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q44 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D45 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A46 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q47 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q48 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E49 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D50 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F51 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F54 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q55 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W57 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A58 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E59 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A60 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G62 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y64 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P66 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R68 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D69 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K70 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P71 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D72 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P74

replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W76 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K77 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R78 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N79 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F80 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R81 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L84 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N85 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R86 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K87 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E88 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G89 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L90 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R91 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L92 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A93 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E94 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D95 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R96 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K98 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D99 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P100 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; H101 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D102 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P103 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; H104 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K105 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y107 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E108 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F109 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N111 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S112 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G113 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G115 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D116

replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F117 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S118 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q119 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P120 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D121 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T122 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S123 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P124 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D125 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N127 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G128 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G130 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T132 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S133 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D134 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q136 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E137 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D138 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I139 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L140 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D141 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E142 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L144 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N146 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V148 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P151 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P153 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D154 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P155 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G156 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P157 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P158 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V,

N, Q, F, W, Y, or C; S159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L160 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A161 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V162 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P164 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E165 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P166 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C167 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P168 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q169 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R172 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S173 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P174 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S175 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L176 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D177 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N178 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P179 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T180 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P181 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F182 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P183 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N184 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G186 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P187 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S188 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E189 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N190 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P191 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L192 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K193 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R194 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L196 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V197 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P198 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E200 replaced with H, K, R, A,

G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E201 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W202 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E203 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F204 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E205 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V206 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A208 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F209 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y210 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R211 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R213 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q214 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F216 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q217 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q218 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T219 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C222 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P223 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E224 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L226 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R227 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L228 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S231 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E232 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D235 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R236 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L238 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P239 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W241 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P242 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N,

Q, F, W, Y, or C; V243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L245 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P246 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D247 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P248 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L252 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D254 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R255 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M258 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y260 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V261 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R262 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H263 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V264 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C267 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L268 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G269 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L272 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A273 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L274 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W275 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R276 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A277 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q279 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; W280 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W282 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A283 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q284 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R285 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

G287 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H288 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C289 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; H290 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T291 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y292 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W293 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A294 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V295 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S296 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E297 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E298 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L299 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P301 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N302 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S303 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G304 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H305 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G306 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P307 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D308 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G309 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E310 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V311 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P312 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K313 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D314 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K315 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E316 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G318 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V319 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F320 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D321 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L322 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G323 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P324 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F325 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I326 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V327 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D328 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L329 replaced with D, E, H, K, R, N, Q, F,

W, Y, P, or C; I330 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T331 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F332 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T333 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E334 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G335 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S336 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G337 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R338 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S339 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P340 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R341 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y342 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A343 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L344 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W345 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F346 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; C347 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; V348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G349 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E350 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W352 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P353 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q354 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D355 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q356 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P357 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W358 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T359 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K360 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R361 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L362 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V363 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M364 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V365 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K366 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V367 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V368 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P369 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T370 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C371 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L372 replaced

with D, E, H, K, R, N, Q, F, W, Y, P, or C; R373 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A374 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L375 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V376 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E377 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M378 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A379 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R380 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V381 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G382 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G383 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A384 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S385 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S386 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L387 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E388 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N389 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T390 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V391 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D392 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L393 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H394 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I395 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S396 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N397 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S398 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H399 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P400 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L401 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S402 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L403 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T404 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S405 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D406 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q407 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y408 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K409 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A410 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y411 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L412 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q413 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D414 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L415 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V416

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E417 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G418 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M419 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D420 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F421 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q422 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G423 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P424 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G425 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E426 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; and/or S427 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting IRF3 proteins of the invention may be routinely screened for IRF3 functional activities and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility) described throughout the specification and known in the art. Preferably, the resulting proteins of the invention have an increased and/or a decreased IRF3 functional activity. More preferably, the resulting IRF3 proteins of the invention have more than one increased and/or decreased IRF3 functional activity and/or physical property.

[0154] To improve or alter the characteristics of IRF3 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0155] Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., Carter *et al., Nucl. Acids Res. 13*:4331 (1986); and Zoller *et al., Nucl. Acids Res. 10*:6487 (1982)), cassette mutagenesis (see e.g., Wells *et al., Gene 34*:315 (1985)), restriction selection mutagenesis (see e.g., Wells *et al., Philos. Trans. R. Soc. London SerA 317*:415 (1986)).

[0156] Thus, the invention also encompasses IRF3 derivatives and analogs that have

one or more amino acid residues deleted, added, or substituted to generate IRF3 polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognitions sequences in the IRF3 polypeptides of the invention, and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the IRF3 at the modified tripeptide sequence (see, e.g., Miyajimo *et al., EMBO J* 5(6):1193-1197). Additionally, one or more of the amino acid residues of the polypeptides of the invention (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins.

[0157] The polypeptides of the present invention include a polypeptide comprising, or alternatively, consisting of: amino acids 1 to 427 in Figure 1 (SEQ ID NO:2); amino acids 2 to 427 in Figure 1 (SEQ ID NO:2); the IRF3 DNA binding domain; the IRF3 nuclear export signal; the IRF3 interferon regulatory domain; the intracellular domain of IRF3; and the IRF3 extracellular domain and the IRF3 intracellular domain with all or part of the transmembrane domain deleted; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above (e.g., the polypeptide of Figure 1 (SEQ ID NO:2)), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 or at least 100 amino acids. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0158] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of an IRF3 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the IRF3 transcription factor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a

reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0159] As a practical matter, whether any particular polypeptide is at least 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to, for instance, the amino acid sequence shown in Figure 1 (SEQ ID NO:2), can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[0160] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (*Comp. App. Biosci.* *6*:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of

the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0161] The present application is also directed to proteins cotaining polypeptides at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the IRF3 polypeptide sequence set forth as n^1-m^1. In preferred embodiments, the application is directed to proteins comprising or alternatively consisting of, polypeptides at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific IRF3 N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0162] In certain preferred embodiments, IRF3 proteins of the invention comprise fusion proteins as described above wherein the IRF3 polypeptides are those described as n^1-m^1, herein. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0163] In preferred embodiments, the IRF3 polypeptide or fragment thereof stimulates transcription from promoters containing IRF3 binding sites (e.g., ISRE or PRDI-PRDIII elements which can be found, for example, in the promoters of ISG15, the chemokine RANTES, or IFN-alpha or IFN-beta genes). The ability of an IRF3 polypeptide (e.g., fragment) to stimulate transcription from promoters containing IRF3 binding sites (e.g., ISRE or PRDI-PRDIII elements) can routinely be determined using techniques described herein or otherwise known in the art. In another non-exclusive preferred embodiment, IRF3 polypeptide or fragment thereof inhibits transcription from promoters containing IRF3 binding sites The ability of an IRF3 polypeptide to antagonize transcription from promoters containing IRF3 binding sites (e.g., ISRE or PRDI-PRDIII elements) can routinely be determined using techniques described herein or otherwise known in the art. For example reporter assays like those described in Shafer et al., J. Biol. Chem. 273:2714 (1998) can be used to measure the ability of an IRF3 polypeptide, fragment, or variant thereof to stimulate, inhibit or not significantly alter transcription from promoters containing IRF3 binding sites.

[0164] In preferred embodiments, the IRF3 polypeptide or fragment interacts with other transcription factors (e.g., the IRF7 and RelA transcription factors). The ability of an IRF3 polypeptide (e.g., fragment) to interact with other transcription can routinely be determined using techniques described herein or otherwise known in the art. In another non-exclusive preferred embodiment, the IRF3 polypeptide or fragment interacts with other transcription factors. The ability of an IRF3 polypeptide to interact with other transcrition factors can routinely be determined using techniques described herein or otherwise known in the art. For example, co-immunopreciptation experiments may be used to determine the interaction between two proteins.

[0165] In one embodiment, one or more of the IRF3 polypeptides of the invention are expressed at relatively high levels in mature T cells. In another embodiment, one or more of the IRF3 polypeptides of the invention are expressed at relatively high levels in macrophages, monocytes, dendritic cells, and/or B cells.

[0166] In one embodiment, the trancription assay described in the paragraph above may be modified for use in screening for an IRF3 related proteins or an agonist or antagonist thereof. In this instance, a baseline level of transcription from promoters containing IRF3 binding sites (e.g., ISRE or PRDI-PRDIII elements) is determined as described above. Potential agonists, antagonists or IRF3 related polypeptide(s) are added to an experimental well and the resultant level of transcription from promoter(s) containing IRF3 binding sites is assessed and compared to the baseline level (where the the baseline level is taken as the amount of transcription from promoter(s) containing IRF3 binding sites in the absence of potential, agonists, antagonists, or IRF3 related polypeptide(s). An increase as the amount of transcription from promoter(s) containing IRF3 binding sites in the experimental well will indicate that the potential IRF3 related proteins(s) or polypeptide(s) is either (or both) an IRF3 related protein or an agonist, whereas a decrease in as the amount of transcription from promoter(s) containing IRF3 binding sites will indicate that the potential IRF3 protein(s) or polypeptide(s) is an antagonist.

Gene Therapy using IRF3 Polynucleotides for the Treatment of Infectious Disease

[0167] In has been discovered, in accordance with the present invention (See Example 1), that HIV replication is blocked in cells of the immune system, more specifically T cells, which overexpress IRF3. Based on this result, it is believed that IRF3 polynucleotides and IRF3 polypeptides as well as fragments thereof will be useful in the treatment of infectious diseases, particularly infectious diseases caused by viruses, and even more particularly AIDS. In a preferred embodiment, the polynucleotides of the invention are used in gene therapy methods to treat infectious diseases, espcially AIDS and other diseases caused by viruses.

[0168] The polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by expression of such polypeptides

in vivo, which is often referred to as "gene therapy." For more detail on this aspect of the invention, see below and Examples 1-3.

[0169] In a specific embodiment, nucleic acids comprising sequences encoding IRF3 polypeptides, fragments or variants, are administered as a form of gene therapy to treat, inhibit or prevent a disease or disorder. In a preferred embodiment, IRF3 nucleic acids comprising sequences encoding IRF3 polypeptides, fragments or variants, are administered as a form of gene therapy to prevent, treat or ameliorate an infectious disease, especially diseases caused by viral infections, though prevention, treatment and/or amelioration of infectious diseases caused by bacterial, fungal, and/or parasitic infections are also encompassed by the invention. In highly preferred embodiments, nucleic acids encoding IRF3 polypeptides, fragments or variants, are administered as a form of gene therapy to prevent, treat or ameliorate diseases and disorder associated with HIV infection, especially AIDS. In another specific embodiment, nucleic acids comprising sequences encoding IRF3 polypeptides, fragments or variants, are administered as a form of gene therapy to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0170] Viruses that cause infectious diseases that may be treated by administration of nucleic acids comprising sequences encoding IRF3 polypeptides, fragments or variants, include, but are not limited to, retroviruses (*e.g.*, human T-cell lymphotrophic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (*e.g.*, herpes simplex virus (HSV) types I and II, Epstein-Barr virus, HHV6-HHV8, and cytomegalovirus), arenavirues (*e.g.*, lassa fever virus), paramyxoviruses (*e.g.*, morbillivirus virus, human respiratory syncytial virus, mumps, and pneumovirus), adenoviruses, bunyaviruses (*e.g.*, hantavirus), cornaviruses, filoviruses (*e.g.*, Ebola virus), flaviviruses (*e.g.*, hepatitis C virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (*e.g.*, hepatitis B viruses (HBV)), orthomyoviruses (*e.g.*, influenza viruses A, B and C), papovaviruses (*e.g.*, papillomavirues), picornaviruses (*e.g.*, rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (*e.g.*, rotavirues), togaviruses (*e.g.*, rubella virus), rhabdoviruses (*e.g.*, rabies virus).

[0171] Bacteria that cause infectious diseases that may be treated by administration of nucleic acids comprising sequences encoding IRF3 polypeptides, fragments or variants, include, but are not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae* , *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromotis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter* (Vibrio) *fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Treponema pallidum*, *Treponema pertenue*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma spp.*, *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia spp.*, and *Helicobacter pylori*.

[0172] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0173] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[0174] In a preferred aspect, the compound comprises nucleic acid sequences encoding an IRF3 polypeptide, fragment, or variants (including, for example fusion protein of the invention), said nucleic acid sequences being part of expression vectors that express the IRF3 polypeptides, fragments, or variants in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the polypeptide coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the polypeptide coding

sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the polypeptide encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989).

[0175] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0176] Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art and are apparent from the teachings herein. For example, cells may be engineered by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention. Cells which may be engineered for use in methods of preventing, treating, or ameliorating viral infection, particularly HIV infection, include, but are not limited to hematopoietic stem cells, T cells, monocytes, macrophages, and dendritic cells.

[0177] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises

a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[0178] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an polypeptide of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the polypeptide to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[0179] Similarly, cells may be engineered in vivo for expression of a polypeptide in vivo by, for example, procedures known in the art. For example, a packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells in vivo and expression of the polypeptide in vivo. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention.

[0180] Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian

leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

[0181] The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller et al., Biotechniques, 7(9) :980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and .beta.-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

[0182] The nucleic acid sequence encoding the polypeptide of the present invention is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the .beta.-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

[0183] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PES01, PA317, .psi.-2, .psi.-AM, PA12, T19-14X, VT-19-17-H2, .psi.CRE, .psi.CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO.sub.4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0184] The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

[0185] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0186] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0187] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0188] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried

out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0189] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0190] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0191] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0192] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an polypeptide are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992);

Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[0193] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

[0194] In a specific embodiment the molynucleotides used in the methods of gene therapy encodes and anti-IRF3 antibody (e.g., an intrabody) as defined and described in the section of this application entitled "Antibodies," below.

Antibodies

[0195] Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind an IRF3 polypeptide, polypeptide fragment, or variant of SEQ ID NO:2, and/or an IRF3 epitope (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In specific embodiments, the immunoglobulin molecules of the invention are IgG1. In other specific embodiments, the immunoglobulin molecules of the invention are IgG4.

[0196] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in

combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

[0197] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0198] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[0199] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated

using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10^{-2} M, 10^{-2} M, 5 X 10^{-3} M, 10^{-3} M, 5 X 10^{-4} M, 10^{-4} M, 5 X 10^{-5} M, 10^{-5} M, 5 X 10^{-6} M, 10^{-6}M, 5 X 10^{-7} M, 10^{7} M, 5 X 10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10^{-9} M, 10^{-9} M, 5 X 10^{-10} M, 10^{-10} M, 5 X 10^{-11} M, 10^{-11} M, 5 X 10^{-12} M, $^{10-12}$ M, 5 X 10^{-13} M, 10^{-13} M, 5 X 10^{-14} M, 10^{-14} M, 5 X 10^{-15} M, or 10^{-15} M.

[0200] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0201] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferrably, antibodies of the present invention bind an antigenic epitope disclosed herein (e.g., amino acid 4-8, 28-33, 66-71, 94-105, 118-128,

132-136, 153-157, 165-168, 173-178, 186-193, 198-202, 233-238, 304-316, 334-340, and 423-427), or a portion thereof.

[0202] The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0203] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0204] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0205] The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment

does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0206] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0207] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0208] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 6). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0209] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0210] Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of Current Protocols in Immunology, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference herein. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Investigators may also choose to perform selection procedures that will enrich the sample for B cells that are antigen-reactive. For example, one method of enriching for antigen-reactive B cells is panning on a plastic dish that has been coated with antigen. Antigen reactive B cells may then be eluted from the plastic dish and used for transformation. Alternatively, it is possible to enrich for antigen-reactive B cells using fluorescence activated cells sorting (FACS). In this method, one might use fluorescently

labelled antigen to sort out a population of antigen reactive B-cells from non-antigen reactive B cells an other cells types. Both FACS analysis and panning, may also be performed ina manner so as to enrich for B cells as opposed to antigen-reactive B cells. The advantage of selecting for total B cells populations is that one is more likely to include plasma cells, or B cells actively secreteing immunoglobulin, that might be missed in procedures that require the presence of cell-surface immunoglobulin for detection. Growth of EBV-infected cells is promoted by monocytes, so investigators may wish to take care not to exclude these form culture, or to resupply monocytes after selection procedures. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

[0211] In general, the sample containing human B cells is innoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g, SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

[0212] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2

fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0213] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0214] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al.,

Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[0215] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO

98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0216] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0217] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

[0218] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to DNA or to another polypeptide can be used to generate anti-idiotypes that "mimic" the binding domain and, as a consequence, bind to and neutralize polypeptide and/or th epolypeptide it ineteracts with. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

[0219] Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen *et al., Hum. Gene Ther.* *5*:595-601 (1994); Marasco, W.A., *Gene Ther.* *4*:11-15 (1997); Rondon and Marasco, *Annu. Rev. Microbiol.* *51*:257-283 (1997); Proba *et al., J. Mol. Biol.* *275*:245-253 (1998); Cohen *et al., Oncogene* *17*:2445-2456 (1998); Ohage and Steipe, *J. Mol. Biol.* *291*:1119-1128 (1999); Ohage *et al., J. Mol. Biol.* *291*:1129-1134 (1999); Wirtz and Steipe, *Protein Sci.* *8*:2245-2250 (1999); Zhu *et al., J. Immunol. Methods* *231*:207-222 (1999); and references cited therein. In particular, a CCR5 intrabody has been produced by Steinberger *et al., Proc. Natl. Acad. Sci. USA* *97*:805-810 (2000).

Polynucleotides Encoding Antibodies

[0220] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an

antibody, preferably, that specifically binds to an IRF3 polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2.

[0221] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0222] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0223] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a

different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0224] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0225] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0226] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be

adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

[0227] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques. Methods of producing antibodies include, but are not limited to, hybridoma technology, EBV transformation, and other methods discussed herein as well as through the use recombinant DNA technology, as discussed below.

[0228] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0229] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0230] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

[0231] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0232] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0233] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of

the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0234] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e,g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0235] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0236] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0237] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0238] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availabilty of cell

lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suplliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al., Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

[0239] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0240] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0241] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

[0242] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP

307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

[0243] As discussed, supra, the polypeptides corresponding to an IRF3 polypeptide, polypeptide fragment, or a variant of SEQ ID NO:2 may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:2 may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[0244] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification

include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0245] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (^{121}I, ^{123}I, ^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), technetium (^{99}Tc,^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, and ^{97}Ru.

[0246] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Y, ^{117}Tin, ^{186}Re, ^{188}Re and ^{166}Ho. In specific

embodiments, an antibody or fragment thereof is attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, ^{177}Lu, ^{90}Y, ^{166}Ho, and ^{153}Sm, to polypeptides. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the an antibody of the invention or fragment thereof via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999) which are hereby incorporated by reference in their entirety.

[0247] A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0248] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See,

International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.,* *6*:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), CD40-ligand, a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0249] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0250] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0251] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0252] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

[0253] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al., Cell, 96*:737-49 (1999)).

[0254] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

[0255] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0256] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol)

supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0257] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0258] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of

the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0259] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Demonstration of Therapeutic or Prophylactic Activity

[0260] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in

which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

[0261] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, such as, for example, an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0262] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0263] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also

be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0264] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0265] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[0266] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0267] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of

its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0268] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W.

Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0269] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0270] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0271] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0272] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0273] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

[0274] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0275] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual

may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0276] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0277] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0278] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[0279] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0280] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0281] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0282] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet

another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[0283] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0284] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0285] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0286] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0287] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0288] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0289] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

<u>Immune System-Related Disorder Diagnosis</u>

[0290] For a number of immune system-related disorders, substantially altered (increased or decreased) levels of IRF3 gene expression may be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IRF3 gene expression level, that is, the IRF3 expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, which involves measuring the expression level of the gene encoding the IRF3 polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard IRF3 gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder or normal activation, proliferation, differentiation, and/or death.

[0291] In particular, it is believed that certain tissues in mammals with cancer of cells or tissue of the immune system express significantly enhanced or reduced levels of the IRF3 polypeptide and mRNA encoding the IRF3 polypeptide when compared to a corresponding "standard" level. Further, it is believed that enhanced or depressed levels of the IRF3 polypeptide can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) or cells or tissue from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

[0292] Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, including cancers of this system, and immunodeficiencies and/or autoimmune diseases which involves measuring the expression level of the gene encoding IRF3 polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard IRF3 gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

[0293] Where a diagnosis of a disorder in the immune system, including, but not limited to, diagnosis of a tumor, diagnosis of an immunodeficiency, and/or diagnosis of an autoimmune disease, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced

or depressed IRF3 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0294] By analyzing or determining the expression level of the gene encoding the IRF3 polypeptide is intended qualitatively or quantitatively measuring or estimating the level of the IRF3 polypeptide or the level of the mRNA encoding the IRF3 polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the IRF3 polypeptide level or mRNA level in a second biological sample). Preferably, the IRF3 polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard IRF3 polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard IRF3 polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0295] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains IRF3 polypeptide or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the IRF3 polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the IRF3. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0296] The compounds of the present invention are useful for diagnosis, prognosis, or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include, but are not limited to tumors (e.g., B cell and monocytic cell leukemias and lymphomas) and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases (e.g., rheumatoid arhtritis, systemic lupus erythamatosus, Sjogren syndrome, mixed connective tissue disease, and inflammatory myopathies), and graft versus host disease.

[0297] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method

described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the IRF3 polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0298] Assaying IRF3 polypeptide levels in a biological sample can occur using antibody-based techniques. For example, IRF3 polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105*:3087-3096 (1987)). Other antibody-based methods useful for detecting IRF3 polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0299] Techniques known in the art may be applied to label polypeptides (including antibodies) of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0300] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the IRF3 (such as, for example, cells of B cell lineage and the spleen). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may

be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the IRF3 gene.

[0301] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of IRF3 gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0302] The antibodies (or fragments thereof) or IRF3 polynucleotides or polypeptides, may additionally be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of IRF3 gene products or conserved variants or peptide fragments thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or IRF3 polypeptide of the present invention. The antibody (or fragment) or IRF3 polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the IRF3 gene product, or conserved variants or peptide fragments, or IRF3 polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0303] Immunoassays and non-immunoassays for IRF3 gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of identifying IRF3 gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0304] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled an anti-IRF3 antibody or detectable polypeptide. The solid phase support may then be washed

with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0305] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0306] The binding activity of a given lot of anti-IRF3 antibody or IRF3 polypeptide may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0307] In addition to assaying IRF3 polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, IRF3 polypeptides or polynucleotides can also be detected in vivo by imaging. For example, in one embodiment of the invention, IRF3 polypeptide and/or anti-IRF3 antibody is used to image B cell lymphomas. In another embodiment, IRF3 polypepitdes and/or anti-IRF3 antibodies and/or IRF3 polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of IRF3 mRNA) is used to image lymphomas (e.g., monocyte and B cell lymphomas).

[0308] With respect to antibodies, one of the ways in which the anti-IRF3 antibody can be detectably labeled is by linking the same to an enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay,

CRC Press, Boca Raton, FL,; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0309] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect IRF3 through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0310] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave-length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0311] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0312] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is

then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0313] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling include, but are not limited to, luciferin, luciferase and aequorin.

Treatment of Infectious Diseases and Immune System-Related Disorders

[0314] The present invention is further directed to IRF3 based therapies which involve administering IRF3 based therapeutic compounds of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the diseases, disorders, or conditions disclosed herein. Therapeutic compounds of the invention include, but are not limited to, IRF3 polypeptides (including fragments and variants of IRF3 polypeptides), polynucleotides encoding these polypeptides, and antibodies that bind these polypeptides. In preferred embodiments, therapeutic compounds of the invention are used to prevent treat or ameliorate infectious diseases, inlcuiding infectious diseases caused by bacteria, fungi, parasites and viruses. In more preferred embodiments, therapeutic compounds of the invention are used to prevent treat or ameliorate infectious diseases caused by viruses. Therapeutic IRF3 compounds of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein. Examples of viruses that cause infections which may be prevented treated or ameliorated by administration of therapeutic compounds of the invention include, but are not limited to, retroviruses (*e.g.*, human T-cell lymphotrophic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (*e.g.*, herpes simplex virus (HSV) types I and II, Epstein-Barr virus, HHV6-HHV8, and cytomegalovirus), arenavirues (*e.g.*, lassa fever virus), paramyxoviruses (*e.g.*, morbillivirus virus, human respiratory syncytial virus, mumps, and pneumovirus), adenoviruses, bunyaviruses (*e.g.*, hantavirus), cornaviruses, filoviruses (*e.g.*, Ebola virus), flaviviruses (*e.g.*, hepatitis C

virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (*e.g.*, hepatitis B viruses (HBV)), orthomyoviruses (*e.g.*, influenza viruses A, B and C), papovaviruses (*e.g.*, papillomavirues), picornaviruses (*e.g.*, rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (*e.g.*, rotavirues), togaviruses (*e.g.*, rubella virus), rhabdoviruses (*e.g.*, rabies virus).

[0315] In highly preferred embodiments, therapeutic compounds of the invention are used to prevent, treat or ameliorate diseases associated by HIV infection, especially AIDS.

[0316] Bacteria that cause infectious diseases that may be treated by administration of therapeutic compounds of the invention include, but are not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae* , *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromotis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter* (Vibrio) *fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Treponema pallidum*, *Treponema pertenue*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma spp.*, *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia spp.*, and *Helicobacter pylori*.

[0317] As noted above, IRF3 polynucleotides and polypeptides (e.g., IRF3 extracellular domain-Fc fusion proteins), and anti-IRF3 antibodies, are useful for diagnosis of conditions involving abnormally high or low expression of IRF3 activities. For example, given the cells and tissues where IRF3 is expressed as well as the activities modulated by IRF3, it is readily apparent that a substantially altered (increased or decreased) level of expression of IRF3 in an individual compared to the standard or "normal" level may produce pathological conditions related to the bodily system(s) in which IRF3 is expressed and/or is active.

[0318] In one embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing IRF3

polypeptides or anti-IRF3 antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells. IRF3 polypeptides (e.g., soluble IRF3 extracellular domain or fragments thereof) or anti-IRF3 antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

[0319] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., IRF3 polypeptides or anti-IRF3 antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0320] IRF3 polynucleotides or polypeptides of the invention, or agonists of IRF3 (e.g., anti-IRF3 agonistic antibodies), can be used in the treatment of infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively IRF3 polynucleotides or polypeptides of the invention, or agonists of IRF3 (e.g., anti-IRF3 agonistic antibodies), may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0321] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by IRF3 polynucleotides or polypeptides of the invention, or agonists of IRF3 (e.g., anti-IRF3 agonistic antibodies). Examples of viruses, that can be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to one or more of the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae,

Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. IRF3 polynucleotides or polypeptides, or agonists or antagonists of IRF3, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, IRF3 polynucleotides or polypeptides, or agonists of IRF3 are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment IRF3 polynucleotides, polypeptides, or agonists are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment, IRF3 polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose AIDS. In an additional specific embodiment IRF3 polynucleotides, polypeptides, agonists, and/or antagonists are used to treat, prevent, and/or diagnose patients with cryptosporidiosis.

[0322] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by IRF3 polynucleotides or polypeptides, or agonists or antagonists of IRF3, include, but not limited to, one or more of the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria (e.g., Listeria monocytogenes), Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae

(e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. IRF3 polynucleotides or polypeptides, or agonists or antagonists of IRF3, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, IRF3 polynucleotides, polypeptides, or agonists thereof are used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

[0323] Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by IRF3 polynucleotides or polypeptides, or agonists or antagonists of IRF3, include, but not limited to, a member of one or more of the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. IRF3 polynucleotides or polypeptides, or agonists or antagonists of IRF3, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, IRF3 polynucleotides, polypeptides, or agonists thereof are used to treat, prevent, and/or diagnose malaria.

[0324] In another embodiment, IRF3 polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose inner ear infection (such as, for example, otitis media), as well as other infections characterized by infection with *Streptococcus pneumoniae* and other pathogenic organisms.

[0325] In a specific embodiment, IRF3 polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-IRF3 antibodies) are used to treat or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, IRF3 polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-IRF3 antibodies) may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pheumocystis carnii.

[0326] IRF3 polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or conditions associated therewith: primary immuodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[0327] Additionally, IRF3 polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions associated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable mutliple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g., neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (e.g., cytamegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogammaglobulinemic neonates with risk

factor for infection or morbidity), epilepsy (e.g., intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (e.g., decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[0328] Additional preferred embodiments of the invention include, but are not limited to, the use of IRF3 polynucleotides, IRF3 polypeptides, and functional agonists or antagonists thereof, in the following applications:

[0329] A vaccine adjuvant that enhances immune responsiveness to specific antigen. In a specific embodiment, the vaccine is an IRF3 polypeptide described herein. In a specific embodiment, the vaccine adjuvant is an IRF3 polypeptide described herein. In another specific embodiment, the vaccine adjuvant is a polynucleotide described herein (e.g., an IRF3 polynucleotide genetic vaccine adjuvant). For example, A DNA vaccine may comprise a polynucleotide encoding an IRF3 polypeptide, fragment or variant and a polynucleotide encoding a paricular antigen. The IRF3-polynucleotide may be administered on the same or separate DNA molecule as the polynucleotide encoding the vaccine antigen. In one embodiment, an IRF-3 genetic adjuvant for use in DNA immunizations is useful for promoting CD8+ T cell responses. In another embodiment, an IRF-3 genetic adjuvant for use in DNA immunizations is useful for promoting CD4+ T cell responses. In another embodiment, an IRF-3 genetic adjuvant for use in DNA immunizations is useful for promoting humural immune responses. As discussed herein, IRF3 polynucleotides may be administered using techniques known in the art, including but not limited to, liposomal delivery, recombinant vector delivery, injection of naked DNA, and gene gun delivery.

[0330] An adjuvant to enhance tumor-specific immune responses.

[0331] An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include, but are not limited to, virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus,

Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to the HIV gp120 antigen.

[0332] An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: Vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphi, Meisseria meningitidis, Streptococcus pneumoniae, Group B streptococcus, Shigella spp., Enterotoxigenic Escherichia coli, Enterohemorrhagic E. coli, Borrelia burgdorferi, and Plasmodium (malaria).

[0333] An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria).

Formulations and Administration

[0334] The IRF3 polypeptide composition (preferably containing anti-IRF3 antibody or a polypeptide which is a soluble form of the IRF3 extracellular domain) will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with IRF3 polypeptide alone), the site of delivery of the IRF3 polypeptide composition, the method of administration, the scheduling of administration, and other factors known to

practitioners. The "effective amount" of IRF3 polypeptide for purposes herein is thus determined by such considerations.

[0335] As a general proposition, the total pharmaceutically effective amount of IRF3 polypeptide administered parenterally per dose will be in the range of about 1 microgram/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day.

[0336] In another embodiment, the IRF3 polypeptide of the invention is administered to a human at a dose betweeen 0.0001 and 0.045 mg/kg/day, preferably, at a dose between 0.0045 and 0.045 mg/kg/day, and more preferably, at a dose of about 45 microgram/kg/day in humans; and at a dose of about 3 mg/kg/day in mice.

[0337] If given continuously, the IRF3 polypeptide is typically administered at a dose rate of about 1 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0338] The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0339] In a specific embodiment, the total pharmaceutically effective amount of IRF3 polypeptide administered parenterally per dose will be in the range of about 0.1 microgram/kg/day to 45 micrograms/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.1 microgram/kg/day, and most preferably for humans between about 0.01 and 50 micrograms/kg/day for the protein. IRF3 polypepitdes of the invention may be administered as a continuous infusion, multiple dicreet injections per day (e.g., three or more times daily, or twice daily), single injection per day, or as discreet injections given intermitently (e.g., twice daily, once daily, every other day, twice weekly, weekly, biweekly, monthly, bimonthly, and quarterly). If given continuously, the IRF3 polypeptide is typically administered at a dose rate of about 0.001 to 10 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump.

[0340] Effective dosages of the compositions of the present invention to be administered may be determined through procedures well known to those in the art which

address such parameters as biological half-life, bioavailability, and toxicity. Such determination is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0341] Bioexposure of an organism to IRF3 polypeptide during therapy may also play an important role in determining a therapeutically and/or pharmacologically effective dosing regime. Variations of dosing such as repeated administrations of a relatively low dose of IRF3 polypeptide for a relatively long period of time may have an effect which is therapeutically and/or pharmacologically distinguishable from that achieved with repeated administrations of a relatively high dose of IRF3 for a relatively short period of time.

[0342] Using the equivalent surface area dosage conversion factors supplied by Freireich, E. J., et al. (*Cancer Chemotherapy Reports* 50(4):219-44 (1966)), one of ordinary skill in the art is able to conveniently convert data obtained from the use of IRF3 in a given experimental system into an accurate estimation of a pharmaceutically effective amount of IRF3 polypeptide to be administered per dose in another experimental system. Experimental data obtained through the administration of IRF3 in mice may converted through the conversion factors supplied by Freireich, et al., to accurate estimates of pharmaceutically effective doses of IRF3 in rat, monkey, dog, and human. The following conversion table (Table III) is a summary of the data provided by Freireich, et al. Table III gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent surface area dose expressed as mg/kg in another species tabulated.

Table III. Equivalent Surface Area Dosage Conversion Factors.

	--*TO*--				
	Mouse	Rat	Monkey	Dog	Human
--*FROM*--	(20g)	(150g)	(3.5kg)	(8kg)	(60kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	5/3	1	1/2
Human	12	7	3	2	1

[0343] Thus, for example, using the conversion factors provided in Table III, a dose of 50 mg/kg in the mouse converts to an appropriate dose of 12.5 mg/kg in the monkey because (50 mg/kg) x (1/4) = 12.5 mg/kg. As an additional example, doses of 0.02, 0.08, 0.8, 2, and 8 mg/kg in the mouse equate to effect doses of 1.667 micrograms/kg, 6.67 micrograms/kg, 66.7 micrograms/kg, 166.7 micrograms/kg, and 0.667 mg/kg, respectively, in the human.

[0344] Pharmaceutical compositions containing IRF3 polypeptides of the invention may be administered orally, rectally, parenterally, subcutaneously, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray (e.g., via inhalation of a vapor or powder). In one embodiment, "pharmaceutically acceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, “pharmaceutically acceptable” means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. Nonlimiting examples of suitable pharmaceutical carriers according to this embodiment are provided in “Remington’s Pharmaceutical Sciences” by E.W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can be

employed as liquid carriers, particularly for injectable solutions. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0345] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0346] In a preferred embodiment, IRF3 compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered subcutaneously.

[0347] In another preferred embodiment, IRF3 compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered intravenously.

[0348] For parenteral administration, in one embodiment, the IRF3 polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0349] Generally, the formulations are prepared by contacting the IRF3 polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0350] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine

or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, sucrose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; preservatives, such as cresol, phenol, chlorobutanol, benzyl alcohol and parabens, and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0351] The IRF3 polypeptide is typically formulated in such vehicles at a concentration of about 0.001 mg/ml to 100 mg/ml, or 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml or 1-10 mg/ml, at a pH of about 3 to 10, or 3 to 8, more preferably 5-8, most preferably 6-7. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of IRF3 polypeptide salts.

[0352] IRF3 polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic IRF3 polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0353] IRF3 polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous IRF3 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized IRF3 polypeptide using bacteriostatic Water-for-Injection.

[0354] Alternatively, IRF3 polypeptide is stored in single dose containers in lyophilized form. The infusion selection is reconstituted using a sterile carrier for injection.

[0355] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally, associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the

present invention may be employed in conjunction with other therapeutic compounds.

[0356] Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0357] In addition to soluble IRF3 polypeptides, IRF3 polypeptides containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as CHAPS or NP-40, with buffer.

[0358] IRF3 compositions of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0359] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0360] Sustained-release compositions also include liposomally entrapped compositions of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing IRF3 polypeptide may be prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800

Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal a polypeptide therapy.

[0361] In another embodiment systained release compositions of the invention include crystal formulations known in the art.

[0362] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0363] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0364] The compositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, compositions of the invention are administered in combination with alum. In another specific embodiment, compositions of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the compositions of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the compositions of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in

combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0365] In another specific embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated therewith. In one embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, compositions of the invention are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0366] The compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to, antiretroviral agents, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0367] In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the

Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0368] Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β-L-FD4C and β-L-FddC (WO 98/17281).

[0369] Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and

DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

[0370] Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myres Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Welcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

[0371] Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

[0372] Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES , NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine recpetor agonists such as RANTES, SDF-1, MIP-1α, MIP-1β, etc., may also inhibit fusion.

[0373] Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an

oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

[0374] Additional antiretroviral agents include hydroxyurea-like compunds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors sucha as VX-497 (Vertex); and myvopholic acids such as CellCept (mycophenolate mofetil; Roche).

[0375] Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

[0376] Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1α, MIP-1β, SDF-1α, IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN-α2a; antagonists of TNFs, NFκB, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al., PNAS 94*:11567-72 (1997); Chen *et al., Nat. Med. 3*:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF-α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α-

naphthoflavone (WO 98/30213); and antioxidants such as γ-L-glutamyl-L-cysteine ethyl ester (γ-GCE; WO 99/56764).

[0377] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat, prevent, and/or diagnose AIDS and/or to treat, prevent, and/or diagnose HIV infection.

[0378] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the compositions of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™,

and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0379] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0380] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin,

chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0381] In preferred embodiments, the compositions of the invention are administered in combination with interferons, including but not limited to interferon-alpha, interferon-beta, and/or interferon-gamma.

[0382] In one embodiment, the compositions of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (International Publication Number WO 97/33902; J. Exp. Med. 188(6):1185-1190) (1998)), endokine-alpha (International Publication No. WO 98/07880), Neutrokine-alpha (Internatioanl Application Publication No. WO 98/18921), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), 312C2 (International Publication No. WO 98/06842), TR12, TACI (See, e.g., U.S. Patent No. 5,969,102; and von Bulow et al., Science 278:138-141 (1997)), CD154, CD70, and CD153.

[0383] In a preferred embodiment, the compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), bioloigically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0384] In a preferred embodiment, the compositions of the invention are administered in combination with TACI (See e.g., U.S. Patent No. 5,969,102; and von Bulow et al.,

Science 278:138-141 (1997)), a soluble form of TACI, biologically active fragments, variants, or derivatives of TACI (e.g., TACI-Fc), and/or anti-TACI antibodies (e.g., agonistic or antagonistic antibodies).

[0385] In a preferred embodiment, the compositions of the invention are administered in combination with Neutrokine-alpha (International Publication No. WO 98/18921), a soluble form of Neutrokine alpha, biologically active fragments, variants, or derivatives of Neutrokine-alpha, and/or anti-Neutrokine alpha antibodies (e.g., agonistic or antagonistic antibodies).

[0386] In a preferred embodiment, the compositions of the invention are administered in combination with APRIL (International Publication Number WO 97/33902; J. Exp. Med. 188(6):1185-1190 (1998)), a soluble form of APRIL, biologically active fragments, variants, or derivatives of APRIL, and/or anti-APRIL antibodies (e.g., agonistic or antagonistic antibodies).

[0387] In a preferred embodiment, the compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0388] In a preferred embodiment, the compositions of the invention are administered in combination with an NSAID.

[0389] In a nonexclusive embodiment, the compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasermin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0390] In a preferred embodiment, the compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California) and prednisolone.

[0391] In a more preferred embodiment, the compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the compositions of the invention are administered in combination with methotrexate. In another embodiment, the compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the compositions of the invention are administered in combination ENBREL™. In another embodiment, the compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specfic embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specfic embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0392] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the

compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0393] CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0394] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the compositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0395] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In another embodiment, compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the compositions of the invention are administered in combination with IL4 and IL10.

[0396] In one embodiment, the compositions of the invention are administered in combination with one or more chemokines. In specific embodiments, the compositions of the invention are administered in combination with an α(CxC) chemokine selected from

the group consisting of gamma-interferon inducible protein-10 (γIP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO-α, GRO-β, GRO-γ, neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a β(CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1γ), macrophage inflammatory protein-3 alpha (MIP-3α), macrophage inflammatory protein-3 beta (MIP-3β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the γ(C) chemokine, lymphotactin.

[0397] In another embodiment, the compositions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the compositions of the invention are administered with chemokine beta-8.

[0398] In an additional embodiment, the compositions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0399] In an additional embodiment, the compositions of the invention are administered in combination with an IL-13 antagonist. IL-13 antagonists that may be administered with the compositions of the invention include, but are not limited to: soluble IL-13 receptor polypeptides, multimeric forms of soluble IL-13 receptor polypeptides;

anti-IL-13 receptor antibodies that bind the IL-13 receptor without transducing the biological signal elicited by IL-13, anti-IL-13 antibodies that block binding of IL-13 to one or more IL-13 receptors, and muteins of IL-13 that bind IL-13 receptors but do not transduce the biological signal elicited by IL-13. Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0400] The invention also encompasses combining the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) with other proposed or conventional hematopoietic therapies. Thus, for example, the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) can be combined with compounds that singly exhibit erythropoietic stimulatory effects, such as erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, and triiodothyzonine. Also encompassed are combinations of the compositions of the invention with compounds generally used to treat aplastic anemia, such as, for example, methenolene, stanozolol, and nandrolone; to treat iron-deficiency anemia, such as, for example, iron preparations; to treat malignant anemia, such as, for example, vitamin B_{12} and/or folic acid; and to treat hemolytic anemia, such as, for example, adrenocortical steroids, e.g., corticoids. See e.g., Resegotti et al., Panminerva Medica, 23:243-248 (1981); Kurtz, FEBS Letters, 14a:105-108 (1982); McGonigle et al., Kidney Int., 25:437-444 (1984); and Pavlovic-Kantera, Expt. Hematol., 8(supp. 8) 283-291 (1980), the contents of each of which are hereby incorporated by reference in their entireties.

[0401] Compounds that enhance the effects of or synergize with erythropoietin are also useful as adjuvants herein, and include but are not limited to, adrenergic agonists, thyroid hormones, androgens, hepatic erythropoietic factors, erythrotropins, and erythrogenins, See for e.g., Dunn, "Current Concepts in Erythropoiesis", John Wiley and Sons (Chichester, England, 1983); Kalmani, Kidney Int., 22:383-391 (1982); Shahidi, New Eng. J. Med., 289:72-80 (1973); Urabe et al., J. Exp. Med., 149:1314-1325 (1979); Billat et al., Expt. Hematol., 10:133-140 (1982); Naughton et al., Acta Haemat, 69:171-179 (1983); Cognote et al. in abstract 364, Proceedings 7th Intl. Cong. of Endocrinology (Quebec City, Quebec, July 1-7, 1984); and Rothman et al., 1982, J. Surg. Oncol., 20:105-108 (1982). Methods for stimulating hematopoiesis comprise administering a

hematopoietically effective amount (i.e., an amount which effects the formation of blood cells) of a pharmaceutical composition containing polynucleotides and/or poylpeptides of the invention (and/or agonists or antagonists thereof) to a patient. The polynucleotides and/or polypeptides of the invention and/or agonists or antagonists thereof is administered to the patient by any suitable technique, including but not limited to, parenteral, sublingual, topical, intrapulmonary and intranasal, and those techniques further discussed herein. The pharmaceutical composition optionally contains one or more members of the group consisting of erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, triiodothyzonine, methenolene, stanozolol, and nandrolone, iron preparations, vitamin B_{12}, folic acid and/or adrenocortical steroids.

[0402] In an additional embodiment, the compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0403] In an additional embodiment, the compositions of the invention are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0404] Additionally, the compositions of the invention may be administered alone or in combination with other therapeutic regimens, including but not limited to, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

Agonists and Antagonists - Assays and Molecules

[0405] The invention also provides a method of screening compounds to identify those which enhance or block the action of IRF3 polypeptide on cells, such as its interaction with IRF3 binding molecules such as ligand molecules. An agonist is a compound which increases the natural biological functions of IRF3 or which functions in a manner similar to IRF3 while antagonists decrease or eliminate such functions.

[0406] In another embodiment, the invention provides a method for identifying a ligand protein or other ligand-binding protein which binds specifically to IRF3 polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds IRF3. The preparation is incubated with labeled IRF3 and complexes of ligand protein bound to IRF3 are isolated and characterized according to routine methods known in the art. Alternatively, the IRF3 interacting polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

[0407] In the assay of the invention for agonists or antagonists, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds IRF3 such as a molecule of a signaling or regulatory pathway modulated by IRF3. The preparation is incubated with labeled IRF3 in the absence or the presence of a candidate molecule which may be an IRF3 agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of IRF3 on binding the IRF3 binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to IRF3 are agonists.

[0408] By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating IRF3 biological activity. IRF3 agonists are useful in increasing the anti-HIV response mediated by IRF3, as described above.

[0409] By "antagonist is intended naturally occurring and synthetic compounds capable of inhibiting or abolishing IRF3 biological activity.

[0410] Another method involves screening for compounds which inhibit or enhance IRF3 biological activity by determining, for example, the amount of transcription from promoters containing IRF3 binding sites in a cell that expresses IRF3. Such a method may involves transfecting a eukaryotic cell with DNA encoding IRF3 such that the cell expresses IRF3, contacting the cell with a candidate agonist or antagonist compound, and determining the amount of transcription from promoters containing IRF3 binding sites. A reporter gene (.e.g, the chloramphenicol transferase (CAT) gene) linked to a promoter

containing an IRF3 binding site may be used in such a method, in which case, the amount of transcription from the reporter gene may be measured by assaying the level of reporter gene product, or the level of activity of the reporter gene product in the case where the reporter gene is an enzyme. An increase in the amount of transcription from promoters containing IRF3 binding sites in a cell expressing IRF3, compared to a cell that is not expressing IRF3, would indicate that the candidate compound is an IRF3 agonist. A decrease in the amount of transcription from promoters containing IRF3 binding sites in a cell expressing IRF3, compared to a cell that is not expressing IRF3, would indicate that the candidate compund is an IRF3 antagonist.

[0411] Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a interferon. The method involves contacting cells which express the IRF3 polypeptide with a candidate compound and an interferon, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of IRF3 and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of IRF3. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or an interferon (e.g., determining or estimating an increase or decrease transcrition from promoters containing IRFs, or an increase or decrese in a expression of a gene product under the control of a promoter element containing an IRF3 binding site). By the invention, a cell expressing the IRF3 polypeptide can be contacted with either an endogenous or exogenously administered interferon.

[0412] Potential agonists include small organic molecules, peptides, and polypeptides. Potential antagonists also may be small organic molecules, a peptide, a polypeptide or oligonucleotide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a ligand molecule, without inducing IRF3 induced activities, thereby preventing the action of IRF3 by excluding IRF3 from binding.

[0413] Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J.

Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the extracellular domain of the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of IRF3. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into IRF3 polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of IRF3.

[0414] In one embodiment, the IRF3 antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the IRF3 antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others know in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding IRF3, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci.

U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

[0415] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of an IRF3 gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded IRF3 antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with an IRF3 RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0416] Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of IRF3 shown in Figure 1, respectively, could be used in an antisense approach to inhibit translation of endogenous IRF3 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of IRF3 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0417] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The

oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., Proc. Natl. Acad. Sci. 84:648-652 (1987); PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0418] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0419] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0420] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a

phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0421] In yet another embodiment, the antisense oligonucleotide is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res. 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res. 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1997)).

[0422] Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451 (1988)), etc.

[0423] While antisense nucleotides complementary to the IRF3 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

[0424] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequence can be used to destroy IRF3 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence IRF3 (Figure 1). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the IRF3 mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0425] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express IRF3 *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous IRF3 messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0426] Endogenous gene expression can also be reduced by inactivating or "knocking out" the IRF3 gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0427] By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and

inducing and/or blocking the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), FasL, CD40L, (TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (International Publication Number WO 97/33902; J. Exp. Med. 188(6):1185-1190) (1998)), endokine-alpha (International Publication No. WO 98/07880), Neutrokine-alpha (International Publication No. WO 98/18921), CD27L, CD30L, 4-lBBL, OX40L, CD27, CD30, 4-1BB, OX40, and nerve growth factor (NGF). In specific embodiments, the TNF-family ligand is Neutrokine-alpha, or fragments or variants thereof. In other specific embodiments, the TNF-family ligand is APRIL or fragments or variants thereof.

[0428] Antagonists of the present invention also include antibodies specific for TNF-family ligands or the IRF3 polypeptides of the invention. Antibodies according to the present invention may be prepared by any of a variety of standard methods using IRF3 immunogens of the present invention. As indicated, such IRF3 immunogens include the complete IRF3 polypeptide depicted in Figure 1 (SEQ ID NO:2) and IRF3 polypeptide fragments comprising, for example, the DNA binding domain, nuclear export signal, interferon regulatory factor association domain, phosphorylation domain, and/or autoinhibitory domain, or any combination thereof.

[0429] Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed herein and/or known in the art, such as, for example, those methods described in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992)); Tartaglia et al., *Cell* 73:213-216 (1993)), and PCT Application WO 94/09137 and are preferably specific to (i.e., bind uniquely to polypeptides of the invention having the amino acid sequence of SEQ ID NO:2.

[0430] In a preferred method, antibodies according to the present invention are mAbs. Such mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow et al., *Antibodies*: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In:

Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13 (Burdon et al., eds.), Elsevier, Amsterdam (1984)).

[0431] The techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of IRF3 thereby effectively generating agonists and antagonists of IRF3. *See generally*, International Publication No. WO 99/29902, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten *et al., Curr. Opinion Biotechnol. 8*:724-33 (1997); Harayama, *Trends Biotechnol. 16*(2):76-82 (1998); Hansson *et al., J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques 24*(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of IRF3 polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired IRF3 molecule by homologous, or site-specific, recombination. In another embodiment, IRF3 polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of IRF3 may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0432] Proteins and other compounds which bind the IRF3 domains are also candidate agonists and antagonists according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two- hybrid system has been described by Roger Brent and his colleagues (Gyuris, *Cell* 75:791-803 (1993); Zervos et al., *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to the DNA binding domain, nuclear export signal, interferon regulatory factor association domain, phosphorylation domain, and the autoinhibitory domain of IRF3. Such compounds are good candidate agonists and antagonists of the present invention.

[0433] For example, using the two-hybrid assay described above, the DNA binding domain or interferon regulatory factor association domain of the IRF3, or a portion thereof, may be used to identify cellular proteins which interact with IRF3 the receptor *in*

vivo. Such an assay may also be used to identify ligands with potential agonistic or antagonistic activity of IRF3 transcription factor function. This screening assay has previously been used to identify protein which interact with the cytoplasmic domain of the murine TNF-RII and led to the identification of two receptor associated proteins. Rothe et al., *Cell* 78:681 (1994).

[0434] Other screening techniques include the use of cells which express the polypeptide of the present invention (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science*, 246:181-296 (1989). In another example, potential agonists or antagonists may be contacted with a cell which expresses the polypeptide of the present invention and a second messenger response, e.g., signal transduction may be measured to determine whether the potential antagonist or agonist is effective.

[0435] Agonists according to the present invention include naturally occurring and synthetic compounds such as, for example, interferon family polypeptides and peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science* 267:1457-1458 (1995)).

[0436] Preferred agonists are fragments of IRF3 polypeptides of the invention which stimulate lymphocyte (e.g., B cell) proliferation, differentiation and/or activation. Further preferred agonists include polyclonal and monoclonal antibodies raised against the IRF3 polypeptides of the invention, or a fragment thereof.

[0437] In an additional embodiment, immunoregulatory molecules such as, for example, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha, may be used as agonists of IRF3 polypeptides of the invention which stimulate leukocyte (e.g., B cell) proliferation, differentiation and/or activation.

[0438] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells

(e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

[0439] Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

[0440] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

[0441] In yet another embodiment of the invention, the activity of IRF3 polypeptide can be reduced using a "dominant negative." To this end, constructs which encode, for example, defective IRF3 polypeptide, such as, for example, mutants lacking all or a portion of the DNA binding domain, can be used in gene therapy approaches to diminish the activity of IRF3 on appropriate target cells. For example, nucleotide sequences that direct host cell expression of IRF3 polypeptide in which all or a portion of the DNA binding domain is altered or missing can be introduced into monocytic cells or other cells or tissues (either by in vivo or ex vivo gene therapy methods described herein or otherwise

known in the art). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous IRF3 gene in monocytes. The engineered cells will express non-functional IRF3 polypeptides .

Diagnostic Assays

[0442] The compounds of the present invention are useful for diagnosis or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include but are not limited to tumors (e.g., B cell and monocytic cell leukemias and lymphomas) and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, and graft versus host disease.

[0443] For a number of immune system-related disorders, substantially altered (increased or decreased) levels of IRF3 gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IRF3 gene expression level, that is, the IRF3 expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a system disorder, which involves measuring the expression level of the gene encoding the IRF3 polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard IRF3 gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of an immune system disorder or normal activation, proliferation, differentiation, and/or death.

[0444] In particular, it is believed that certain immune cells in mammals express significantly reduced levels of normal or altered IRF3 polypeptide and mRNA encoding the IRF3 polypeptide when compared to a corresponding "standard" level are susceptible to viral infection, including, in particular HIV infection. Further, it is believed that enhanced or depressed levels of the IRF3 polypeptide can be detected in certain immune cell types or tissue from mammals with such a susceptibility when compared to cellsfrom mammals of the same species not having the susceptibility.

[0445] Thus, the invention provides a diagnostic method useful during diagnosis of a susceptibility to viral infection, including HIV infection, which involves measuring the

expression level of the gene encoding the IRF3 polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard IRF3 gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an susceptibility to viral infection.

[0446] Where a diagnosis of a disorder in the immune system, including diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed IRF3 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0447] By “assaying the expression level of the gene encoding the IRF3 polypeptide” is intended qualitatively or quantitatively measuring or estimating the level of the IRF3 polypeptide or the level of the mRNA encoding the IRF3 polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the IRF3 polypeptide level or mRNA level in a second biological sample). Preferably, the IRF3 polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard IRF3 polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard IRF3 polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0448] By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing IRF3 transcription factor protein (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the IRF3 polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the IRF3 transcription factor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0449] Total cellular RNA can be isolated from a biological sample using any suitable

technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the IRF3 polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0450] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of IRF3 transcription factor protein, or the soluble form thereof, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of IRF3, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as an IRF3 protein of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying IRF3 protein levels in a biological sample can occur using any art-known method.

[0451] Assaying IRF3 polypeptide levels in a biological sample can occur using antibody-based techniques. For example, IRF3 polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105*:3087-3096 (1987)). Other antibody-based methods useful for detecting IRF3 polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0452] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the IRF3 gene or cells or tissue which are known, or suspected, to express the IRF3 interacting protein gene. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane

(Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the IRF3 gene or IRF3 interacting protein gene.

[0453] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of IRF3 gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0454] The antibodies (or fragments thereof), and/or IRF3 polypeptides, of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of IRF3 gene products or conserved variants or peptide fragments thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or IRF3 polypeptide of the present invention. The antibody (or fragment) or IRF3 polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the IRF3 gene product, or conserved variants or peptide fragments, or IRF3 polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0455] Immunoassays and non-immunoassays for IRF3 gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding IRF3 gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0456] Immunoassays and non-immunoassays for IRF3 interacting protein gene

products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectable or labeled IRF3 polypeptide capable of identifying IRF3 interacting protein gene products or conserved variants or peptide fragments thereof, and detecting the bound IRF3 polypeptide by any of a number of techniques well-known in the art.

[0457] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled anti-IRF3 antibody or detectable IRF3 polypeptide. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0458] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0459] The binding activity of a given lot of anti-IRF3 antibody or IRF3 polypeptide may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0460] In addition to assaying IRF3 polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, IRF3 polypeptide or polynucleotide can

also be detected in vivo by imaging. For example, in one embodiment of the invention, IRF3 polypeptide is used to image monocytic leukemias or lymphomas. In another embodiment, IRF3 polynucleotides of the invention and/or anti-IRF3 antibodies (e.g., polynucleotides complementary to all or a portion of IRF3 mRNA) are used to image B cell leukemias or lymphomas.

[0461] Antibody labels or markers for *in vivo* imaging of IRF3 polypeptide include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of IRF3 polypeptide for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al., Nature 314*:268 (1985).

[0462] Additionally, any IRF3 polypeptide whose presence can be detected, can be administered. For example, IRF3 polypeptides labeled with a radio-opaque or other appropriate compound can be administered and visualized in vivo, as discussed, above for labeled antibodies. Further such IRF3 polypeptides can be utilized for in vitro diagnostic procedures.

[0463] AN IRF3 polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the

case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain IRF3 protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0464] With respect to antibodies, one of the ways in which the anti-IRF3 antibody can be detectably labeled is by linking the same to an enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0465] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect IRF3 through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is

incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0466] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0467] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0468] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0469] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Chromosome assays

[0470] The nucleic acid molecules of the present invention are also valuable for chromosome identification.

[0471] In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of an IRF3 transcription factor gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA is then used for *in situ* chromosome mapping using well known techniques for this purpose.

[0472] In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

[0473] Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al., Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

[0474] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0475] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

[0476] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1

Method of Treatment Using Gene Therapy –Ex vivo

[0477] It has been discovered, in accordance with the present invention, that overexpression of IRF3 can block HIV replication in vitro. The Jurkat T cell line was transfected with IRF3 expressing plasmid together with a plasmid encoding the selective marker gene (neomycin) and the transfected cells were selected by their ability to grow in

G418. The surviving clones were examined for IRF3 expression. The clone which showed the highest levels of IRF3, as detected by western blot, was selected for further studies.

[0478] The IRF3 expressing clone was then infected with a laboratory strain of HIV-1, which uses CxCR4 as a co-receptor. Virus replication was followed over the course of 14 days and compared to the HIV-1 replication in the parental Jurkat cell line. The results of two independent analyses have shown that in the cells that over-express IRF3, viral replication is blocked.

[0479] We have further analyzed whether the inhibition of HIV-1 replication is due to the decrease in number of HIV-1 receptors in these cells. The number of CD4 and CxCR receptors expressed on these cells and untransfected control cells was comparable. Also, over-expression of IRF3 in these cells did not alter the growth rate of these cells. By way of non-limiting hypothesis, it is possible that overexpression of IRF3 results in increased levels of autocrine chemokine expression, which may contribute to blocking viral replication by inhibiting virus spreading by blocking viral entry into cells. These results indicate that IRF3 (e.g., IRF3 polynucleotides, and IRF3 polypeptides as well as fragments and variants thereof) is an ideal candidate gene that may be used in the treatment of infectious diseases, particularly infectious diseases caused by viruses, and even more particularly AIDS. In particular, because IRF3 is a transcription factor, i.e., an intracellular protein, IRF3 is a good candidate for gene therapy methods to treat infectious diseases, espcially AIDS and other diseases caused by viruses.

[0480] One method of gene therapy transplants immune cells, such as, for example, T cells, monocytes or macrophages, which are capable of expressing IRF3 polypeptides, onto a patient. Several protocols are known in the art for gene therapy. One protocol which has been successful in the treatment of adenosine deaminase deficiency (ADA) is briefly described here. A more detailed descrition of this protocol may be found in Onodera et al., Blood 91:30 (1998) and in Blaese et al., Hum. Gene. Ther. 58:1 (1990). Peripheral T lymphocytes may be obtained from a patient using apheresis using, for example, the CS3000 plus, Baxter Corp. Chicago, IL, and then isolated by density centrifugation. Isolated T cells are then expanded in vitro by growth in AIM-V medium (Gibco, BRL) supplemented with 5% FCS, 100 units/ml recombinant human IL-2 and 10ng.ml anti-CD3 antibody (e.g., Orthoclone OKT3 Injection, Ortho, Raritan, NJ). The

anti-CD3 antibody and recombinant IL-2 treatment activate T cells. The T cells are activated because activated, rather than resting, T cells are more readily transduced by retroviruses. After three days in culture, the cells are infected with a retroviral vector containing a polynucleotide encoding an IRF3 polypeptide of the invention that is operably linked to a promoter. Half of the medium is removed and replaced with medium supplemented with IL-2 and 10micrograms/ml protamine containing the LASN retroviral vector encoding the IRF3 polypetide of the invention. The LASN vector is described more fully in Hock et al., Blood 74:876 (1989) and may prepared and obtained, for example, from a company such as Genetic Therapy, Inc. (Gaithersburg, MD). The transduction procedure is described in Hock et al., Blood 74:876 (1989) and incorporates low temperature (32°C) incubation and centrifugation. After both rounds of transduction, the cells were placed in fresh medium (that did not conatin retrovirus) supplemented with IL-2, and cultured for 6 days. After which the cells are harvested, washed extensively with saline containing 0.5% human albumin, and then are reinfused into the patient.

Example 2

Method of Treatment Using Gene Therapy - In Vivo

[0481] Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) IRF3 sequences into an animal to increase or decrease the expression of the IRF3 polypeptide. The IRF3 polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the IRF3 polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata H. *et al., Cardiovasc. Res. 35*:470-479 (1997); Chao J. *et al., Pharmacol. Res. 35*:517-522 (1997); Wolff J.A. *Neuromuscul. Disord. 7*:314-318 (1997); Schwartz B. *et al., Gene Ther. 3*:405-411 (1996); Tsurumi Y. *et al., Circulation 94*:3281-3290 (1996) (incorporated herein by reference).

[0482] The IRF3 polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The IRF3

polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0483] The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the IRF3 polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P.L., *et al. Ann. NY Acad. Sci. 772*:126-139 (1995), and Abdallah B., *et al. Biol. Cell 85*(1):1-7 (1995)) which can be prepared by methods well known to those skilled in the art.

[0484] The IRF3 polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0485] The IRF3 polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of bone marrow, blood, muscle, skin, brain, lung, liver, spleen, thymus, heart, lymph, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their

ability to take up and express polynucleotides.

[0486] For the naked IRF3 polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked IRF3 polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0487] The dose response effects of injected IRF3 polynucleotide in muscle *in vivo* is determined as follows. Suitable IRF3 template DNA for production of mRNA coding for IRF3 polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

[0488] Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The IRF3 template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

[0489] After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for IRF3 protein expression. A time course for IRF3 protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of IRF3 DNA in muscle

following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using IRF3 naked DNA.

Example 3

Gene Therapy Using Endogenous IRF3 Gene

[0490] Another method of gene therapy according to the present invention involves operably associating the endogenous IRF3 sequence with a promoter via homologous recombination as described, for example, in US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al., Proc. Natl. Acad. Sci. USA 86*:8932-8935 (1989); and Zijlstra *et al., Nature 342*:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired. Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous IRF3, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of IRF3 so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

[0491] The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

[0492] In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be

administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

[0493] Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous IRF3 sequence. This results in the expression of IRF3 in the cell. Expression may be detected by immunological staining, or any other method known in the art.

[0494] Cells that may be transformed include, but are not limited to, hematopoietic cells, and T cells. For example T cells may be obtained from an individual as described in Example 7. Cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately $3X10^6$ cells/ml. Electroporation should be performed immediately following resuspension.

[0495] Plasmid DNA, prepared by standard techniques is added to a sterile cuvette with a 0.4 cm electrode gap (Bio- Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately $1.5.X10^6$ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

[0496] Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37°C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

[0497] The engineered cells are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The engineered cells now produce the protein product and can then be introduced into a patient as described above.

Example 4

Protein Fusions of IRF3

[0498] IRF3 polypeptides of the invention are optionally fused to other proteins. These

fusion proteins can be used for a variety of applications. For example, fusion of IRF3 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See EP A 394,827; Traunecker, *et al., Nature 331*:84-86 (1988)). Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to IRF3 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made using techniques known in the art or by using or routinely modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[0499] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below (SEQ ID NO:3). These primers also preferably contain convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

[0500] For example, if the pC4 (Accession No. 209646) expression vector is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and IRF3 polynucleotide is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

[0501] If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGCCCA
GCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACG
TAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGA

GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
AGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAG
CCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT
(SEQ ID NO:3)

Example 5

Isolation of antibody fragments directed against polypeptides of the present invention from a library of scFvs.

[0502] Naturally occuring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against polypeptides of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

Rescue of the library

[0503] A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harbouring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 ug/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to innoculate 50 ml of 2xTY-AMP-GLU, 2x108 TU of Δ gene 3 helper phage (M13 Δ gene III, see WO92/01047) are added and the culture incubated at 37° C for 45 minutes without shaking and then at 37° C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 minutes and the pellet resuspended in 2 liters of 2xTY containing 100 ug/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[0504] M13 Δ gene III is prepared as follows: M13 Δ gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a

greater avidity of binding to antigen. Infectious M13 Δ gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37° C with shaking. Cells are pelleted (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2xTY broth containing 100 ug ampicillin/ml and 25 ug kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37° C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 um filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the library

[0505] Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 mg/ml or 10 mg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37° C and then washed 3 times in PBS. Approximately 1013 TU of phage are applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37° C. The E. coli are then plated on TYE plates containing 1% glucose and 100 ug/ml ampicillin. The resulting bacterial library is then rescued with Δ gene III helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of binders

[0506] Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then

by sequencing.

Example 6

Production of an anti-IRF3 Antibody

a) Hybridoma Technology

[0507] The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing IRF3 are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of IRF3 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0508] In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with IRF3 polypeptide or, more preferably, with a secreted IRF3 polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

[0509] The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the IRF3 polypeptide.

[0510] Alternatively, additional antibodies capable of binding to IRF3 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the IRF3 protein-specific antibody can be blocked by IRF3. Such antibodies comprise anti-idiotypic antibodies to the IRF3 protein-specific antibody and can be used to immunize an animal to induce formation of further IRF3 protein-specific antibodies.

[0511] It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, secreted IRF3 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

[0512] For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation of antibody fragments directed against IRF3 from a library of scFvs.

[0513] Naturally occuring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against IRF3 to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

[0514] ***Rescue of the Library.*** A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047. To rescue phage displaying antibody fragments, approximately 10^9 E. coli harbouring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 ug/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to innoculate 50 ml of 2xTY-AMP-GLU, 2 x 10^8 TU of delta gene 3 helper (M13 delta gene III, see WO92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of of 2xTY containing 100 ug/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[0515] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harbouring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2xTY broth containing 100 ug ampicillin/ml and 25 ug kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 um filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

[0516] ***Panning of the Library.*** Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 ug/ml or 10 ug/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10^{13} TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized

with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 ug/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

[0517] ***Characterization of Binders.*** Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

Example 7

Method of Detecting Abnormal Levels of IRF3 in a Biological Sample

[0518] IRF3 polypeptides can be detected in a biological sample, and if an increased or decreased level of IRF3 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

[0519] For example, antibody-sandwich ELISAs are used to detect IRF3 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to IRF3, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced using technique known in the art. The wells are blocked so that non-specific binding of IRF3 to the well is reduced.

[0520] The coated wells are then incubated for > 2 hours at RT with a sample containing IRF3. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound IRF3.

[0521] Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The

plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

[0522] 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution is then added to each well and incubated 1 hour at room temperature to allow cleavage of the substrate and flourescence. The flourescence is measured by a microtiter plate reader. A standard curve is preparded using the experimental results from serial dilutions of a control sample with the sample concentration plotted on the X-axis (log scale) and fluorescence or absorbance on the Y-axis (linear scale). The IRF3 polypeptide concentration in a sample is then interpolated using the standard curve based on the measured flourescence of that sample.

Example 8

Method of Treating Decreased Levels of IRF3

[0523] The present invention also relates to a method for treating an individual in need of an increased level of IRF3 biological activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an IRF3 agonist.

[0524] Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of IRF3 in an individual can be treated by administering, for example, an IRF3 agonist, preferably in a soluble and/or secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of IRF3 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of IRF3 agonist to increase the biological activity level of IRF3 in such an individual.

Example 9

Method of Treating Increased Levels of IRF3

[0525] The present invention relates to a method for treating an individual in need of a decreased level of IRF3 biological activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of IRF3 antagonist.

[0526] Antisense technology is used to inhibit production of IRF3. This technology is one example of a method of decreasing levels of IRF3 polypeptide, preferably a soluble and/or secreted form, due to a variety of etiologies, such as cancer.

[0527] For example, a patient diagnosed with abnormally increased levels of IRF3 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the is determined to be well tolerated.

Example 10

Bioassay for the effect of IRF3 polypeptides, agonists, or antagonists on hematopoietic progenitor cells and/or differentiation.

[0528] Mouse bone marrow cells are used as target cells to examine the effect of IRF3 polypeptides of the invention on hematopoietic progenitor cells and/or differentiation. Briefly, unfractionated bone marrow cells are first washed 2X with a serum-free IMDM that is supplemented with 10% (V/V) BIT (Bovine serum albumin, Insulin and Transferrin supplement from Stem Cell Technologies, Vancouver, Canada). The washed cells are then resuspended in the same growth medium and plated in the 96-well tissue culture plate (5×10^4 cells/well) in 0.2 ml of the above medium in the presence or absence of cytokines and transfected with an IRF3 expression vector. Stem cell factor (SCF) and IL-3 may be included as positive mediators of cell proliferation. Cells are allowed to grow in a low oxygen environment (5% CO_2, 7% O^2, and 88% N_2) tissue culture incubator for 6 days. On the sixth day, 0.5 μCi of Tritiated thymidine is added to each well and incubation is continued for an additional 16-18 hours, at which point the cells are harvested. The level of radioactivity incorporated into cellular DNA is determined by scintillation spectrometry and reflects the amount of cell proliferation. Mock transfected, or non-transfected cells should be used to set the baseline level of proliferation.

[0529] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[0530] The entire disclosure of each document cited (including patents, patent

applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

[0531] Further, the Sequence Listing submitted herewith, in both computer and paper forms, is hereby incorporated by reference in its entirety. Additionally, U.S. Non-Provisional Application Serial No. 60/239,936 and U.S. Patent 6,054,289 are herein incorporated by reference in their entirety.